CHEMOTHERAPY OF MALARIA

REVISED SECOND EDITION

L.J. BRUCE-CHWATT, Editor

R.H. BLACK, C.J. CANFIELD,
D.F. CLYDE, W. PETERS,
W.H. WERNSDORFER

World Health Organization Geneva

B.R. Publishing Corporation Delhi

CHEMOTHERAPY OF MALARIA

REVISED SECOND EDITION

CHEMOTHERAPY OF MALARIA

REVISED SECOND EDITION

L J BRUCE-CHWATT, Editor
Emeritus Professor of Tropical Hygiene University of London
formerly Director Ross Institute
London School of Hygiene and Tropical Medicine London England

R H BLACK
Professor of Tropical Medicine Commonwealth Institute of Health University of Sydney Sydney NSW Australia

CRAIG J CANFIELD
Director Division of Experimental Therapeutics Walter Reed Army Institute of Research Washington DC USA

D F CLYDE
Senior Regional Malaria Adviser Who Regional Officer for South East Asia New Delhi India

W PETERS
Professor of Medical Protozoology London School of Hygiene and Tropical Medicine London England formerly Walter Myers Professor of Parasitology Liverpool School of Tropical Medicine Liverpool England

W H WERNSDORFER
Chief Research and Technical Intelligence Malaria Action Programme World Health Organization Geneva Switzerland

World Health Organization, Geneva

B.R. PUBLISHING CORPORATION
DELHI-110007

Sales Office:
D.K. Publishers Distributors (P) Ltd.
1, Ansari Road, Darya Ganj
New Delhi-110002
Phone 3261465, 3278368

WHO Technical Report Series, No 27

ISBN 81-7018-645-5
Code No. C 00566

Printed at
A P Offset, Delhi-110032

CONTENTS

PREFACE

At its fourth session, held in Kampala, Uganda, in December 1950, the WHO Expert Committee on Malaria recommended that information on the properties of antimalarial drugs should be summarized for the benefit of the medical profession. On its advice a drafting committee was appointed to undertake this task, consisting of Sir Gordon Covell (Chairman), Dr G. Robert Coatney, Dr John W Field and Lieutenant-Colonel Jaswant Singh. The members of the drafting committee prepared a text which was reviewed in consultation with a number of experts in this field and published in a WHO monograph entitled *Chemotherapy of malaria* (1955).

Since the publication of this monograph, scientific literature on this subject has increased in both quality and quantity. WHO itself has published reports of scientific groups on malaria chemotherapy and the resistance of plasmodia to antimalarial drugs in its Technical Reports Series. However, the fresh information is widely dispersed through the literature and needs to be brought together for all those in any way involved in the control of malaria. It should be noted, too, that the considerable scientific progress of the past 20 years relates more to knowledge of the effects of existing drugs than to the development of new ones. Research involving the screening and testing of more than 250 000 compounds has shown that only 4 or 5 have a chemotherapeutic index sufficient to justify clinical and field trials. Observations on these compounds have been included in the present monograph.

Chemotherapy has been of great importance since the control of malaria was first attempted. However, the development of residual insecticides somewhat overshadowed the role of antimalarial drugs, particularly in the late 1950s and 1960s. With the dramatic resurgence of malaria in many countries and the increased resistance of vectors to insecticides, antimalarial drugs are regaining their importance, despite the resistance of some strains of *Plasmodium falciparum* to 4-aminoquinolines, particularly in South-East Asia and South America, and to pyrimethamine and proguanil in Africa. This resistance is an additional reason for bringing together in a monograph information on all the available drugs, their use (either alone or in combination), their dosages and regimens for different purposes (whether for prophylaxis, suppression or radical cure), and their toxicity or adverse effects. Such an up-to-date monograph should enable medical practitioners to make the most appropriate selection of antimalarial drugs, either for general use or for the treatment of individual cases.

At a time when governments are considering the development of a primary health care system as a basic function in the achievement of the goal of health for all by the year 2000, this monograph should represent a significant contribution by WHO.[1] It should be of particular importance to African countries south of the Sahara where no organized malaria control programmes can be undertaken on a large scale and where antimalarial drugs are in practice the only effective method of preventing the mortality and reducing the morbidity caused by the disease. Indeed, such drugs, if readily available to the rural population of Africa, would greatly reduce the number of deaths caused by the disease, which is estimated at approximately one million per annum in the child population under five years of age.

I should like to take this opportunity to express my gratitude to the editor and the authors, who have devoted so much of their time to the preparation of this monograph.

H. Mahler, M.D.
Director-General

[1]WHO UNICEF (1978) *Primary health care. Report of the International Conference on Primary Health Care, Alma Ata, USSR, 6–12 September 1978* Geneva, World Health Organization

REVISED SECOND EDITION

DEVELOPMENTS IN MALARIA CHEMOTHERAPY

Since the publication of the second edition of this book in there have been a number of developments in malaria chemotherapy, and these were reviewed by a Scientific Group on the Chemotherapy of Malaria in Geneva in September . The report of this Group[1] emphasizes the detrimental impact of drug resistance of *Plasmodium falciparum* and its further spread, especially in Africa south of the Sahara. It covers, in detail, the results obtained with mefloquine and the combination of mefloquine, sulfadoxine, and pyrimethamine. Both mefloquine alone and the combination have recently been registered and the latter will soon be available for the treatment of multiresistant malaria. The Scientific Group was concerned about maintaining the efficacy of antimalarial drugs and made recommendations for their operational use. As the development of resistance to antimalarial compounds cannot be avoided for ever, there will continue to be a need for new drugs. Phenanthrenemethanols and pyridinemethanols are candidate compounds at an advanced stage of development and hold considerable promise. Artemisinine (qinghaosu) and some of its derivatives may become useful drugs for the emergency treatment of severe falciparum malaria. There are yet other compounds that merit preclinical and clinical development. However, these investigations and the search for new candidate drugs and innovative principles in malaria chemotherapy will largely depend on the intensity of future research.

The chemotherapy of malaria has in recent years assumed a major role in primary health care and in complying with the basic objectives of preventing mortality and curbing morbidity and suffering from malaria. Antimalarial drugs make it possible to pursue these objectives in areas where other antimalarial measures cannot be applied for technical, operational, or financial reasons. It is therefore important to rationalize the use of antimalarial drugs in an effort to maintain their efficacy.

The following sections summarize the developments in malaria chemotherapy that have occurred since the publication of the second edition of

Chemotherapy of malaria. Some of these developments have necessitated a certain number of changes in the main chapters of the book.

Drug Resistance of *P. falciparum*

Chloroquine resistance of *P. falciparum* in eastern Asia and Oceania has shown further consolidation and a significant westward spread. All of Indonesia and practically all areas of India with *P. falciparum* are now affected by chloroquine resistance, which has also been reported from one focus in northern Pakistan. In South America, the situation has largely remained unchanged as far as the geographical distribution of chloroquine-resistant *P. falciparum* is concerned, but there has been a general consolidation in the degree of resistance.

Major and alarming changes have occurred in Africa, south of the Sahara and in islands off the eastern coast. Chloroquine-resistant *P. falciparum* has now been reported from 14 African countries, namely Angola, Burundi, Central African Republic, Comoros, Gabon, Kenya, Madagascar, Malawi, Namibia, Sudan, Uganda, United Republic of Tanzania, Zaire, and Zambia. While chloroquine is still clinically quite useful for the treatment of falciparum malaria in most semi-immune persons, a significant number of RII and even RIII responses have been observed in young children, especially in Malawi, United Republic of Tanzania, and Zambia.

Resistance to the first-line alternative combination, sulfadoxine-pyrimethamine, has also spread and consolidated itself in certain hard-core areas of chloroquine resistance in South America and eastern Asia, such as Brazil, Colombia, Democratic Kampuchea, Thailand, and Viet Nam. In parts of Thailand, for instance, the efficacy of sulfadoxine-pyrimethamine is compromised to a degree that precludes its routine use for the initiation of treatment of falciparum malaria. Resistance of *P. falciparum* to sulfadoxine-pyrimethamine has also been reported from East African countries such as Kenya, United Republic of Tanzania, and Zambia.

Assessment of Drug Response of *P. falciparum*

While a post-treatment observation period of 28 days generally—though not always—suffices to exclude RI responses in the *in vivo* test for chloroquine, a much longer period of 63 days is required for ascertaining S responses to mefloquine. This is because of the long half-life of mefloquine (usually 20–30 days in adults).

The *in vitro* macrotest for mefloquine sensitivity of *P. falciparum* has been phased out and replaced by the microtest, which is now also available for testing sensitivity to amodiaquine and quinine, as well as to chloroquine and mefloquine. A microtest procedure for assessing sensitivity to sulfadoxine-pyrimethamine is currently being developed.

Drug Prophylaxis of Malaria

Detailed recommendations for the drug prophylaxis of malaria in non-immune visitors to malarious areas, non-immune and semi-immune residents of malarious areas, and specific risk groups are given in the report of the Scientific Group on the Chemotherapy of Malaria.[2] Pyrimethamine alone can no longer be recommended for prophylaxis; recent observations with the weekly administration of 200 mg of proguanil in East Africa[3] indicate that this drug has retained a remarkable prophylactic potential against falciparum malaria.

The forthcoming introduction of mefloquine, alone and in combination with sulfadoxine and pyrimethamine, is expected to modify prophylaxis recommendations in areas with multiresistant *P. falciparum*, where, so far, a common practice has been to administer a combination of a 4-aminoquinoline (amodiaquine or chloroquine) and sulfadoxine-pyrimethamine.

Operational Use of Antimalarial Drugs

The operational use of antimalarial drugs was extensively reviewed by the Scientific Group on the Chemotherapy of Malaria in 1983.[2] In view of the considerable selection pressure exerted by mass drug administration (especially if it is repetitive) and the associated risk of promoting and accelerating the occurrence and spread of drug resistance, such mass administration and prophylaxis are no longer recommended. Instead, the use of curative dose regimens is recommended for the treatment of clinical malaria cases, wherever possible on the basis of microscopic diagnosis. The primary health care concept lends itself to such a procedure, which becomes practically mandatory in areas of multiple resistance, when relatively expensive alternative drugs need to be employed. Pregnant women comprise the only group among semi-immune residents of malarious areas for which drug prophylaxis is recommended (from the fourth month of pregnancy to 6 weeks after delivery).

Presumptive treatment (i.e., treatment given to a presumptive malaria case at the time when a blood sample is taken for examination, aimed at relieving symptoms and preventing transmission) should be used only when the delay between blood sampling and administration of curative treatment can be kept below 7 days.

The above considerations preclude the further use of potentially subcurative, low doses of drugs in areas with a relatively high, general immunity, e.g., in tropical Africa. Curative doses are to be used instead, being all the more necessary when the presence of clinical malaria indicates insufficient immunity in the individual patient.

WHO Technical Report Series, No. 711, 1984.
McLARTY, D G ET AL *Lancet*, 2: 656–658 (1984).

Radical Treatment of Vivax and Ovale Malaria

Hypnozoitocidal (antirelapse) treatment of vivax and ovale malaria in adults is usually based on a fortnight's treatment with daily doses of 15 mg of primaquine (base), following the administration of blood schizontocidal therapy with a 4-aminoquinoline. This may cause significant methaemoglobinaemia and haemolysis in subjects deficient in glucose-6-phosphate dehydrogenase (G6PD), who are more effectively treated with *weekly* doses of 45 mg of primaquine for 8 weeks. This regimen is better tolerated than the daily administration of a smaller dose; it can be given without prior G6PD screening and is of advantage in areas where G6PD deficiency occurs frequently.

Treatment of Severe and Complicated Malaria

Recent studies on the management of cerebral malaria have indicated that corticosteroids are contraindicated in the treatment of this condition.[4] Similarly, the use of heparin is unhelpful and may be positively dangerous.[5]

An initial loading dose of quinine of 20 mg/kg of body weight in patients suffering from cerebral malaria and known to be previously untreated permits the establishment of high plasma levels (15–20 mg of quinine/litre) in the acute phase. The efficacy of this treatment outweighs the risks of toxicity, and patients given such a loading dose have a higher chance of survival than those treated in the conventional way.[6] It has been found that the pharmacokinetics of quinine are altered significantly by malaria infection, clearance and apparent volume of distribution being lower during the acute phase of falciparum malaria. Thus a reduction in the quinine dose after clinical improvement (as suggested in the 1981 edition of this book) is inappropriate and it is recommended that quinine be given in 3 daily doses of 10 mg/kg of body weight each for 7–10 days (see Table 7, page 123).

The parenteral administration of quinine (intramuscular or intravenous) in children is strictly contraindicated for toxicological reasons.

Studies by White et al.[7] indicated that quinidine is more effective than quinine. Other, as yet unpublished, observations have confirmed this finding. Where quinine is not available, quinidine may therefore be an acceptable alternative for emergency treatment of severe and complicated malaria. This drug is commonly found in the cardiology department of hospitals. However, particular care should be taken to monitor any cardiotoxic effects of quinidine.

[4] WARRELL, D. A ET AL. *New England journal of medicine*, **306**: 313–319 (1982).
[5] PUNYAGUPTA, S ET AL *American journal of tropical medicine and hygiene*, **23**: 551–559 (1974).
[6] WHITE, N. J. ET AL. *American journal of medicine*, **73**: 564–572 (1982).
[7] WHITE, N. J. ET AL. *Lancet*, **2**: 1069–1071 (1981).

Mefloquine

Following detailed preclinical studies as well as extensive clinical and field trials,[8] both mefloquine and its combination with sulfadoxine and pyrimethamine have been registered in Switzerland. Registration of the combination is pending in several countries where multidrug-resistant *P. falciparum* is a problem.

In experimental models, the combination of sulfadoxine and pyrimethamine with mefloquine proved to delay the emergence of resistance against the latter. Therefore, it is not envisaged to employ or even market mefloquine alone (registered under the name of Lariam) in countries where *P. falciparum* is transmitted. Likewise, it is envisaged that the use of the combination of mefloquine, sulfadoxine, and pyrimethamine (to become available under the trade name of Fansimef) will be largely restricted to the curative treatment of falciparum malaria.

Fansimef tablets contain 250 mg of mefloquine (base), 500 mg of sulfadoxine, and 25 mg of pyrimethamine. A curative dose for adults of normal weight (50–70 kg) consists of 3 tablets of Fansimef administered in a single dose. For adults and children above 5 years of age, the dose should be adjusted on the basis of 12.5 mg of mefloquine (base) per kg of body weight. The medicament is generally well tolerated, but patients treated with Fansimef should be advised to stay in bed for at least 3 days, and preferably 7 days.

Fansimef has not yet been cleared for administration to pregnant women or to children below the age of 5 years, as appropriate clinical observations have not yet been completed.

The Scientific Group on the Chemotherapy of Malaria, recognizing the urgent need to protect mefloquine and ensure its deployment, strongly recommended:

(*a*) that governments should legislate for strict control of the importation, distribution, and utilization of mefloquine alone or in drug combinations;

(*b*) that the use of mefloquine by communities in endemic areas should be restricted to the treatment of acute malaria attacks that are likely to be due to multiple drug-resistant *P. falciparum* in specific groups;

(*c*) that, when available, drug combinations known to delay the development of drug resistance should be used for prophylaxis and treatment instead of mefloquine;

(*d*) that mefloquine should *not* be distributed for use as a single prophylactic drug by residents in endemic areas.

CHAPTER 1

GENERAL

Introduction

The present monograph attempts to retain as much as possible of the outline of the previous edition, but at the same time aims at incorporating most of the substantial changes that the chemotherapy of malaria has undergone during the past two decades, and especially those owed to the appearance of resistance of plasmodia to our most reliable antimalarial drugs.

The task of the authors has been facilitated by the issue by WHO of a number of documents that have periodically reviewed the problems created in the field either by operational difficulties in malaria eradication or by technical obstacles.

The present second edition of the monograph aims primarily at helping the medical profession and public health officers in tropical developing countries where malaria is still prevalent. It is also hoped that it will serve as a handy reference book for medical practitioners in many parts of the world where cases of imported malaria are increasingly common. It should be of value to undergraduate or postgraduate students and provide guidance for those medical auxiliaries who qualify for more advanced training in health care programmes. The ready availability of up-to-date information on the prevention and treatment of malaria should make it easier to offer sound advice to travellers to tropical countries.

Historical Outline

From time immemorial malaria has been one of the most prevalent of human diseases, affecting particularly the populations of tropical regions but also in the past those of temperate climates. It is also one of the oldest infections mentioned in early writings in Egypt, India and China. Its clinical symptoms were fully described by Hippocrates 400 years before the Christian era.

Attempts at treatment by the roots, leaves and flowers of many plants were of little, if any, value, although the powdered roots of Ch'ang shan (*Dichroa febrifuga*), used in China for at least 2000 years, have an undoubted medicinal effect, owing to the presence of an alkaloid, febrifugine, isolated and analysed only recently. Qing hao (*Artemisia annua*), also used for a similar period in

China, has been shown to be a schizontocide of very low toxicity (see page 101). However, the first potent remedy against malaria was discovered only in the seventeenth century following the contact of Europe with the New World.

Although many historians maintain that malaria was introduced into the Americas only after the discovery of the New World by Columbus, there is some evidence that the disease was known to the local populations long before then. Whether the curative virtue of the bark of the "fever trees" growing on the mountainous slopes of the Peruvian Andes was known to the local inhabitants before the Spanish conquest remains uncertain and controversial. The story of the Countess of Chinchón, wife of the Viceroy of Peru, having been cured in 1630 of tertian fever by an administration of an infusion of the bark has been often told in the past. This romantic episode has now been disproved by modern historians, but the latinized and misspelled name of *Cinchona*, given in 1749 by Linnaeus to the "fever tree," became part of our scientific heritage.

The exact date of the introduction of the new remedy into Europe is not known, but it is likely that it was brought to Rome in 1632 by Spanish priests. It became widely used a few years later, thanks to the interest of Cardinal Juan de Lugo, who used it himself for treatment of his fever and then stimulated the distribution of the new drug to missionaries in distant lands. In 1663 Sebastiano Badi (or Bado) in Italy described the medicinal uses of Peruvian bark in various fevers and the powdered preparation became widely used in southern Europe. The connexion of the new drug with the Roman Catholic Church slowed down its use in Protestant England, but when it cured King Charles II's tertian ague it gained greater acceptance and Thomas Sydenham popularized its use. However, religious prejudice against "Jesuits' bark" and the occasional death of patients treated with small amounts of it or with mixtures of other ingredients purporting to be bark created a current of opinion hostile to it. Another factor in its unpopularity was that it was used indiscriminately for the treatment of any febrile disease.

The spectacular cure in 1682 of the French heir to the throne by the English pyretologist Robert Tabor enhanced the popularity of the new remedy all over Europe and beyond. It was soon introduced into India by the British and the Dutch. In 1692 the missionary fathers cured the Chinese Emperor K'ang Hsi of a malignant fever using powdered bark brought from India.

Its therapeutic value was gradually recognized, and by 1677 it was included in the London Pharmacopoeia as *Cortex peruanus*. Although Francesco Torti in Italy insisted in 1712 that cinchona bark was specific only for intermittent fevers, the new remedy suffered from a period of unpopularity owing to erroneous prescribing by many physicians. This lasted until 1765, when James Lind in Calcutta showed that to obtain the best results the drug must be given in full doses. His conclusion that "In the proper administration of the bark the cure of ague may be said to entirely consist" paved the way for wider use of cinchona powder, which eventually became a sovereign fever

remedy. In some countries its success was still doubtful until Maillot, a French physician in Algeria, started using it in large doses with good results.

For 200 years the crude bark was used for the preparation of powders and infusions. Many chemists attempted to isolate the active principle of the drug; it seems that, at the beginning of the nineteenth century, Antonio Gomez in Portugal and Th.I. Gize of Kharkov in Russia obtained a crystalline substance from an alcoholic extract of the bark. But the final isolation of two basic alkaloids of cinchona—quinine and cinchonine—was not accomplished till 1820, by the French chemists Pierre Pelletier and Joseph Caventou. Following the isolation of two other alkaloids of cinchona (quinidine and cinchonidine), factories for the manufacture of various salts of quinine were established in many parts of the world.

The demand for the new drug was so great (especially during the Civil War in the United States of America) that the production of quinine was insufficient to satisfy all the requirements. The exploitation of the native forests of cinchona in Peru was carried out in a most improvident manner. Attempts to develop cinchona plantations in other parts of the world were made by the French in 1743, when de Condamine was sent to Ecuador and Peru, but they ended in failure. The Dutch started the first cinchona cultivation in Java in 1854, thanks to Justus Hasskarl, a botanist who collected the seeds in Bolivia and Peru. In 1872 the British geographer, Clements Markham, established successful plantations in Ceylon and the Nilgiri Hills in India, but his seedlings, as also those of Hasskarl, had a low yield in quinine. Another collector, Charles Ledger, obtained seeds of Bolivian plants of high quality with great difficulty and sold them to the Dutch. From these seeds of *Cinchona ledgeriana* came the best-yielding trees and within 50 years the Dutch plantations of Java were producing 97 % of the world's supply of quinine and had a virtual monopoly, producing in the 1930s about 10 million kg of bark a year.

During the Second World War, in 1943–44, an attempt to increase the production of cinchona bark in South and Central America from seeds transported by air from the Philippines was made by the Americans, but by then the synthetic antimalarials had made their appearance and the demand for quinine was less urgent.

Synthetic antimalarial drugs

The development of synthetic antimalarial drugs forms one of the most interesting chapters in the history of chemotherapy. To understand its successive stages mention must be made of two events that took place concurrently at the end of the nineteenth century. In 1880 Laveran discovered malaria parasites in the blood of man. His discovery stimulated a search for similar organisms in animals and 10 years later Danilevsky found a variety of parasites in the blood of birds. The Russian worker's discovery was published at the same time as Guttmann & Ehrlich's observation that methylene blue

had some beneficial effect on a patient suffering from malaria. Thirty years later these two seemingly unconnected findings were linked together.

The search for synthetic antimalarial drugs stimulated by Perkin's early unsuccessful attempts at producing artificial quinine was pioneered by German chemists. This search would have been impossible without some method of testing the action of new compounds on animal models. Avian malaria provided such a method and in 1926 Roehl modified and standardized the technique used previously by French workers, the brothers Sergent. Roehl's method, using *Plasmodium relictum* in canaries, is the first routine screening test that compared the activities of new compounds in relation to quinine. Other tests using different malaria parasites of birds were introduced at a later stage.

Various attempts at synthesizing quinine were made soon after the isolation of the active principles of cinchona, but all of them failed. Already during the First World War the Germans, finding themselves cut off from the main world supply of quinine in India and Java, had begun to consider the possibility of producing alternative compounds with antimalarial action. The observation made by Ehrlich of the effect of methylene blue was the starting-point of the new venture. Roehl's test provided a method of assessment of the effectiveness of the various compounds synthesized by the chemists.

Another important event that tends to be forgotten was the use, dating from 1918, of malaria therapy for the treatment of neurosyphilis. This led to rapid advances in knowledge of plasmodial infections themselves and of various methods of treatment. The contribution of malariotherapy centres in Britain, France, Italy, Romania, the USSR and the USA has been invaluable.

With Ehrlich's previous observations in mind, Schulemann and his colleagues, Schönhofer and Wingler, first directed their attention in the 1920s to thiazine derivatives related to methylene blue. One of the compounds with a basic dialkylaminoalkylamino side chain was found to be active against avian malaria parasites. Combination of the basic group with a 6-methoxyquinoline, which is the quinoline nucleus of the cinchona alkaloids, led to the first synthetic antimalarial compound of the 8-aminoquinoline series, named Plasmochin (pamaquine). The chemical structure of pamaquine was published only in 1928, but by then a number of British, French and Russian workers had some knowledge of the relationship between the chemical structure of these compounds and their antimalarial action. In the 1930s the French synthesized several homologues of pamaquine and one of them, known as Fourneau 710 or Rhodoquine, became widely known.

Soon after the discovery of pamaquine it became obvious that, while it was highly active against avian plasmodia, it was not so in human malaria; it had a limited action on the asexual forms of *P. falciparum* and toxicity that was by no means negligible. The search for better quinine substitutes continued.

In 1932 Kikuth announced the discovery by Mauss & Mietzsch of a series of compounds synthesized by attaching the basic side chain (evolved for pamaquine) to other heterocyclic compounds. The quinoline ring was

replaced by acridine—a yellow dye, and one of the compounds of the series, originally called Atebrin, proved to have considerable activity on the asexual forms of *P falciparum* About 12 000 different compounds were tested in the course of this work in Germany alone, but a number of related compounds were also prepared in the USSR

The introduction of Atebrin, now called mepacrine (quinacrine in the USA), was delayed for a number of years because of uncertainty about the toxic effect of long-term administration Trials carried out in Algeria, Italy, Malaya, Romania and the USSR under the aegis of the League of Nations confirmed the high suppressive value of mepacrine, but provided no decisive proof of its safety

In the meantime Sinton & Bird in India discovered that pamaquine could greatly reduce the relapse rate of vivax malaria This was of fundamental importance for further studies of the 8-aminoquinolines

The Second World War cut off the Allies from the main sources of quinine in Indonesia, which was occupied by the Japanese Army This created a serious military problem for the Allies, as their forces were engaged in campaigns in some of the most malarious areas of the world Consequently research on synthetic antimalarials received very high priority in Europe and in the USA In order to preserve the supplies of quinine a usable mixture of all the active alkaloids of cinchona, known as Totaquina, was recommended by the Malaria Commission of the League of Nations for wider use. Intensive studies of the absorption, distribution and excretion of mepacrine carried out in Britain and the USA indicated its value for the treatment of acute malaria However, the importance of this drug as a suppressive, when taken for prolonged periods, became obvious only in 1943 1944 as a result of brilliant field studies by Fairley and his team These tests, carried out on nearly 1000 Australian army volunteers, proved that a daily dose of 100 mg of the drug could be continued for months and even years without serious ill-effects. Huge quantities of the new compound were produced in the United Kingdom and the USA Mepacrine was soon introduced for routine use in all the malarious theatres of war and enabled the Allied forces in south-east Asia and the south-west Pacific to maintain their fighting condition There is no exaggeration in saying that this probably changed the course of modern history

The discovery of mepacrine by the Germans had not stopped their attempts to find other and perhaps better antimalarials During their studies the German scientists found that changes in the basic side chain attached in position 4 of the quinoline nucleus produce a series of compounds with good antimalarial properties Two of these compounds of the 4-aminoquinoline series, named Sontochin and Resochin, were synthesized by Andersag as early as 1934, but a few tests did not show that they were superior to mepacrine. Just before the Second World War both compounds were retested by the Germans on cases of human malaria and Sontochin was given preference over Resochin because of its lesser toxicity. In 1941 samples of

these compounds were obtained by the French, who investigated them in Tunisia and confirmed their high activity.

This information was transmitted to the USA, where an extensive programme of chemotherapeutic research had already been launched in 1941. This programme depended on close cooperation between the armed services, scientific institutions, university laboratories and pharmaceutical firms. The chemotherapeutic studies involved the preliminary screening of over 17 000 compounds against several species of avian malaria, the evaluation of the toxicological and pharmacological characteristics of selected compounds in laboratory animals and the final assessment in cases of human malaria, often in volunteers.

In the course of this collective and remarkably coordinated study several derivatives of 4-aminoquinolines were found to be superior to any other drugs.[1] Two of these—chloroquine and amodiaquine—underwent extensive clinical studies in 1944 and the former (corresponding to Resochin) was found to be an outstanding antimalarial compound, faster in therapeutic action than mepacrine or Sontochin and less toxic. Amodiaquine was almost equally effective. The two remained the best therapeutic and suppressive drugs for over 25 years.

In the course of this research programme a number of compounds of the 8-aminoquinoline series were also synthesized and screened. Three promising antimalarials (pentaquine, isopentaquine and primaquine) were found. They differed from pamaquine in the structure of the side chain and had the same, if not a better, effect on relapsing vivax malaria in human subjects. Of these three, primaquine had the lowest toxicity. It remains today the best among comparable compounds for the radical cure of relapsing infections. A closely related compound, named quinocide (chinocid), has been synthesized more recently by Russian scientists.

An intensive research programme on synthetic antimalarials was also conducted during the Second World War in the United Kingdom. The British chemists started with the synthesis of a large series of derivatives of pyrimidine because of the known importance of pyrimidine compounds in cell metabolism. In the course of these studies Curd, Davey & Rose in 1945 obtained a compound of the chloroguanil series which they simplified by opening the pyrimidine ring to produce a biguanide. It was later found that this compound is metabolized in the body, producing a very active form of the drug. Thus proguanil (chlorguanide) was discovered to have an activity on avian malaria greater than that of quinine and with a good margin of safety in laboratory animals. This new drug was given exhaustive clinical trials by the Australian team of Hamilton Fairley at Cairns and proved to be an

[1] The remarkable story of chloroquine from 1934 to 1946, about the involvement of a score of investigators in 6 countries and the initial discovery of the compound, its rejection, rediscovery and field evaluation and the final verdict, has been told by Coatney (1963). It illustrates the pitfalls in the search for and introduction of some chemotherapeutic compounds.

outstanding causal prophylactic agent in falciparum malaria and a satisfactory suppressive of vivax malaria.

Proguanil came into wider use at the end of the Second World War, by when most of the military problems related to a high malaria incidence in tropical areas had become less urgent because of the availability of mepacrine. Nevertheless, the value of the new causal prophylactic drug became firmly established for the prevention of malaria in persons working in the tropics. However, since its action was slow when given for the treatment of acute malaria, and since it seemed to induce drug resistance in some strains of plasmodia, the search for better compounds continued.

The discovery of proguanil, which started with the study of pyrimidines, stimulated further investigation of this group of compounds in the early 1950s. Several 2,4-diaminopyrimidines have the property of inhibiting the growth of lactic acid bacteria through competition with the folic (pteroylglutamic) and folinic acids necessary for their multiplication. The prediction that useful antimalarial compounds could be developed from that series was soon confirmed on bird plasmodia (*P. gallinaceum*), and even more so on the *Plasmodium berghei* of rodents discovered in 1948 by Vincke. The latter discovery was of great value, as it provided the scientists with an experimental animal model of unparalleled simplicity and convenience. The most active of the new compounds, pyrimethamine, developed in 1951 jointly by an American and British team (Falco & Hitchings) was found to be highly effective against human malaria.

The discovery of pyrimethamine was hailed as an important advance, since the new compound had activity similar to but far higher than that of proguanil. It persisted for a long time in the body and there was a remarkably wide margin of safety between the active and the toxic dosage. However, it was soon found that resistance to pyrimethamine appeared relatively rapidly, not only in experimental conditions but also in the field; and cross resistance between pyrimethamine and proguanil was also in evidence. Another closely related compound, trimethoprim, has more recently been shown to exhibit a varying degree of activity against strains of *P. falciparum* resistant to some of the older drugs.

The development of chlorproguanil, with an activity longer than that of its parent compound, was another step in the direction of expanding the range of available antimalarials. The improved reliability of screening methods, such as the testing of antimalarial drugs on *P. cynomolgi* and *P. knowlesi* in rhesus monkeys, perfected by Schmidt in the USA, represented another advance in chemotherapy.

In spite of some drawbacks of the new compounds, it seemed in the 1950s that the arsenal of antimalarial drugs was almost complete. Some workers believed that most of the problems related to the chemotherapy of malaria had been solved, and the generally successful large-scale use of a combination of chloroquine and primaquine in military forces returning home after having been exposed to malaria during the Korean war enhanced the conviction that,

while no single compound approached the ideal in all respects, the range of specific action of several available drugs was sufficient to deal with any malaria situation.

This may have been one of the reasons why, with the exception of a brief burst of enthusiasm generated by the possibility of the incorporation of chloroquine into common salt (the so-called Pinotti method) and the development of injectable repository compounds effective for a few months, chemotherapeutic research in malaria declined markedly in the late 1950s.

A new and menacing event in the history of the chemotherapy of malaria occurred in 1960 with the observation of resistance of *P. falciparum* to chloroquine, which came at a time when the eradication of malaria seemed to be showing good progress.

The concept of malaria eradication, which has been developing since the early 1950s, when residual insecticides were introduced in many fields of public health, was endorsed by WHO and given an integrated plan in 1956. At that time the increasing frequency of reports on the resistance of human plasmodia to proguanil and pyrimethamine caused some disappointment, but the importance of the phenomenon from the point of view of those concerned with malaria eradication seemed relatively small, firstly because during the period of change of strategy from malaria control to malaria eradication the role of drugs was not fully appreciated, and secondly because proguanil and pyrimethamine, particularly valuable for the prevention of infection and for their sporontocidal effect, are rather slow and uncertain therapeutic agents when used for the treatment of overt malaria.

On the basis of previous experimental work and field observations, it was believed that in human plasmodia the development of resistance to the 4-aminoquinolines was unlikely, to say the least. This complacency was shaken when the failure of chloroquine treatment to cure a *P. falciparum* infection originating in Colombia was reported in 1960 by the American workers Young and Moore. *P. falciparum* infections not responding to the usual curative doses of chloroquine were also described in Brazil and Venezuela. Reports on apparent chloroquine resistance soon came from Thailand, Peninsular Malaysia and other countries in south-east Asia, especially southern Viet Nam, where the number of cases occurring in the United States forces caused much concern. Acute falciparum malaria responded to quinine, even though this drug did not always produce a radical cure.

The seriousness of the possibility of widespread resistance of *P. falciparum* to chloroquine—the most widely used drug in the chemotherapy of malaria and a powerful weapon in the service of malaria eradication—was fully recognized by WHO. Particular attention was then paid to careful appraisal of any reports on alleged drug resistance and to the determination of criteria for the recognition of this phenomenon.

The appearance of resistance in some malaria parasites to the 4-aminoquinolines and other available synthetic compounds revealed the relative poverty of the chemotherapeutic arsenal and the narrow margin of

safety in the treatment of disease caused by plasmodia resistant to the best of existing drugs Steps were taken by WHO to assist research in this particular field which in the past decade has extended over three main areas

(*a*) the collection of data and assessment of the distribution degree and other characteristics of drug resistance
(*b*) the study of the biological mechanisms involved and
(*c*) the search for new compounds that could be used as alternative therapeutic agents

Many studies in various countries have been coordinated and sponsored by WHO, but the broadest and most comprehensive scientific effort was launched in 1963 in the United States of America by the US Army Research Program on Malaria Two years later this gigantic programme was well in its stride and various compounds were being screened It was set up when the USA faced the urgent task of protecting and treating its military forces in south-east Asia against the danger of falciparum malaria resistant to the 4 aminoquinolines and other compounds

The availability of rodent and simian experimental models of malaria greatly facilitated the task of screening the new compounds produced by the chemists Moreover since 1965 it has been shown that the three main species of human malaria parasites can be transmitted to the South American owl monkey (*Aotus trivirgatus*) and this advance has substantially extended the scope of experimental studies

At this point the sulfones and sulfonamides re entered the modern history of the chemotherapy of malaria Before the Second World War when the use of sulfonamides revolutionized the treatment of bacterial infections a number of reports had stated that these compounds have an effect on experimental malaria in animals and also on some infections in man However as the action of various sulfones and sulfonamides was slow and variable, this finding was of limited interest Nevertheless the results of investigation into the mode of action of sulfonamides drew attention to the chemical analogues of some metabolites as possible chemotherapeutic agents and to the potentiating action of two compounds acting on different points of the biochemical cycle of growth of the malaria parasite All this was revealed during the study of the biochemical action of proguanil and pyrimethamine in the 1950s Following the discovery of resistance of *P falciparum* to chloroquine, the synergistic action of sulfonamides or sulfones given together with pyrimethamine or proguanil aroused much interest and was of practical value, as shown by the Australians in south-east Asia

The development of long-acting sulfonamides has increased interest in these compounds Sulfamethoxypyridazine, sulfadimethoxine, sulfalene and sulfadoxine have been used (the latter two compounds most commonly), in association with pyrimethamine, for the treatment of falciparum malaria resistant to the 4-aminoquinolines

Dapsone (diaminodiphenyl sulfone) with pyrimethamine or a diformyl derivative of the former were also introduced for the prophylaxis of malaria, but the value of these preparations has not yet been fully assessed. Other compounds or their combinations have been launched, some of them with rather indecent haste. Nevertheless, it is certain that a number of drugs of this series have already found their rightful place as antimalarials.

Some of these and related compounds have been extensively investigated. In the 1960s Thompson devoted much attention to the repository compound cycloguanil embonate, which was thought to be active for a long period after a single injection. Field studies showed that the protection afforded by this and other similar compounds was shorter than expected.

Various other compounds, such as diamidineureas, pyrocatechols, naphthoquinones, 6-aminoquinolines, tetrahydrofurans and quinazolines, have been screened for antiplasmodial activity. Some quinolines and their esters have shown a fair degree of effectiveness in experimental malaria in animals.

Recently several antibiotics have been used experimentally in monkeys and also in patients in south-east Asia, apparently with promising results. In fact, the early trials of antibiotics for malaria go back to the 1950s, when these drugs revolutionized the treatment of syphilis, yaws, relapsing fever, plague and rickettsial infections. Reassessment of these drugs showed that tetracyclines are of some value, but only when administered after a quinine regimen.

The drug screening programme carried out in the USA has been mentioned before. It seems that some of the most promising future drugs for the prevention and treatment of malaria will emerge from this far-seeing enterprise. The programme coordinated by the Walter Reed Army Institute of Research was designed to include the screening of available compounds from various sources and the synthesis of promising new compounds. During the past 12 years over 250 000 compounds have been screened in primary tests using mice infected with *P. berghei*. About 170 of the most active compounds were selected for advanced testing in monkeys infected with simian malaria. Pharmacological and toxicological studies were then carried out on selected compounds, and clinical and field tests on those which showed the greatest promise. By 1974, of the 26 new drugs or their combinations 11 had undergone full trials and of these several have demonstrated high activity against drug-resistant *P. falciparum*. It must be appreciated that the difficulty of trials on cases of human malaria is due to the amount and complexity of preclinical information now required and to the extreme care with which such trials are carried out.

As the result of this comprehensive research programme, 4 new chemical groups have emerged as potentially valuable new antimalarials: (1) 4-quinolinemethanols, (2) 9-phenanthrenemethanols, (3) 2,4-diaminoquinazolines, and (4) 2,4-diaminotriazines. Foremost among the new drugs was a

derivative of a 4-quinolinemethanol(WR 142490), which has now received the generic name of mefloquine.

Extended trials of this compound carried out on naturally infected populations produced good results and, although mefloquine is not yet available for general use, it and other valuable compounds (9-phenanthrene- and 4-pyridinemethanols) are a good augury for further advances in the chemotherapy of malaria generally and for the treatment of drug-resistant falciparum infections in particular.

In the meantime, methods of detecting the presence of chloroquine-resistant strains of *P. falciparum* have been greatly advanced by the use of *in vitro* tests developed by Rieckmann. Their simplicity and convenience are a great advantage in assessing the geographical distribution and degree of resistance, which appears to affect populations everywhere, though at a slower pace than could be expected. Resistance of *P falciparum* to 4-aminoquinolines has now been reported from east Africa, Bangladesh, Burma, China, India, Papua New Guinea, the Philippines, the Solomon Islands, and Vanuatu (formerly New Hebrides), in addition to the previously affected areas of northern South America and south-east Asia, though in most cases only part of the area seems to be marked by this phenomenon.

In spite of the distant promise of a malaria vaccine and some real advances in applied immunology, there can be no doubt that for the foreseeable future we shall depend on the now available and forthcoming chemotherapeutic methods for the prevention and treatment of malaria.

The malaria situation throughout the world (Fig. 1) causes increasing concern. The number of malaria cases in southern Asia and Middle America during the past few years shows a sharp increase; and the number of cases of malaria imported into the countries of the temperate zone has been rising every year, owing to the greater than ever mobility of human populations as well as the deteriorating malaria situation in many developing tropical areas. At the end of 1979 some 2350 million people were living in areas where the transmission of malaria has not ceased; at least one sixth of these people were still living in places where no organized antimalaria measures were being undertaken, especially in Africa south of the Sahara.

These figures show better than any other pointer that global eradication of malaria, however desirable, is an extremely difficult enterprise and that one of today's major tasks is not to lose the gains achieved during past decades.

The history of the chemotherapy of malaria over this century shows the value of close collaboration between fundamental research in academic or other institutions, applied work carried out by the pharmaceutical industry, and field work in which national and international health authorities are involved. Such collaboration offers the best hope for success in fighting one of the world's oldest, most debilitating and most prevalent tropical diseases.

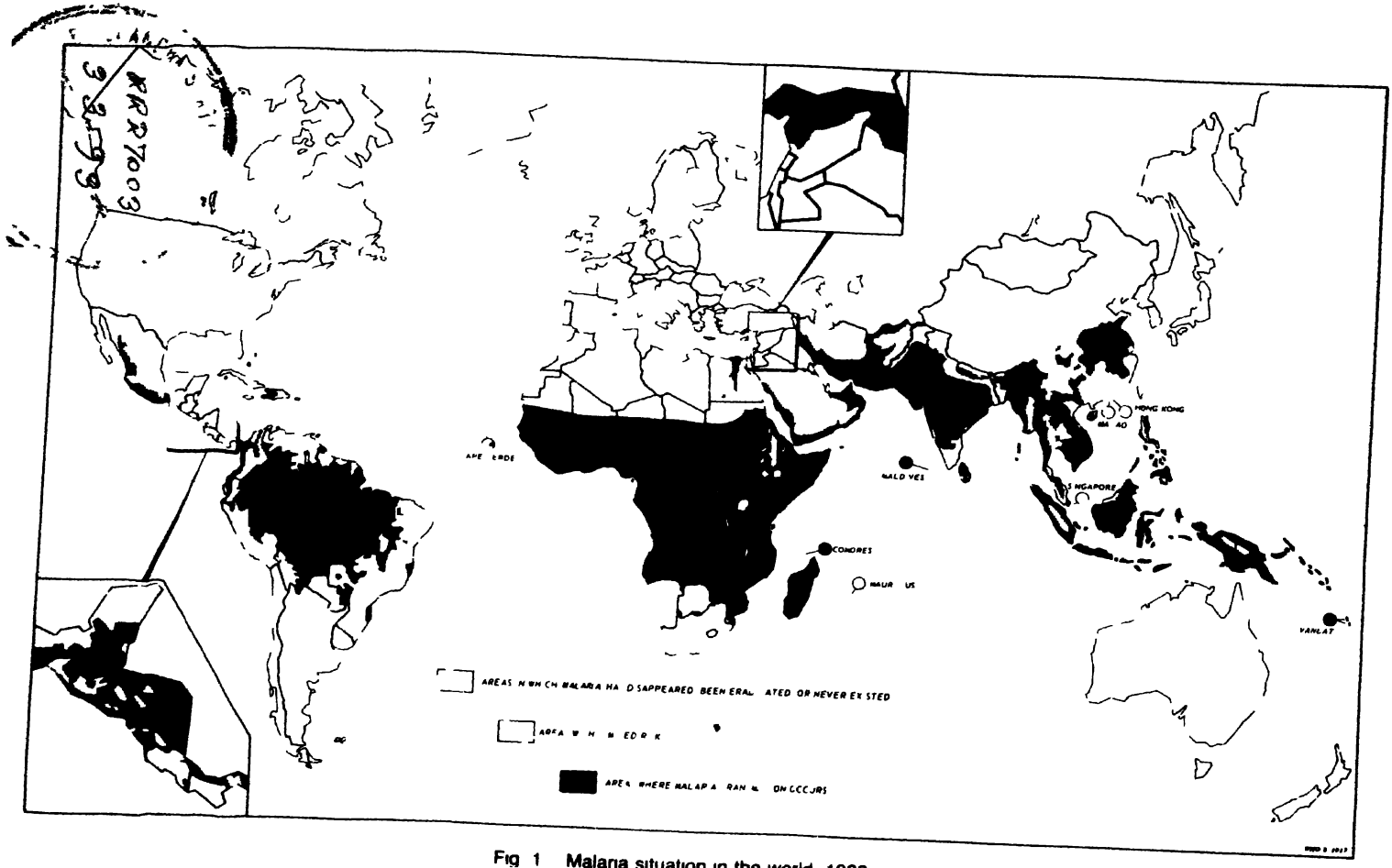


Fig 1 Malaria situation in the world 1980

CHAPTER 2

FUNDAMENTAL ASPECTS OF CHEMOTHERAPY OF MALARIA

Rationale of Malaria Chemotherapy

The principles governing the chemotherapy of malaria are still rooted in the main concepts that guided Paul Ehrlich in his pioneering studies at the end of the nineteenth century. Ehrlich postulated that pathogenic bacteria and parasites possess a whole series of chemoreceptors that differ from one another. Some of these receptors have no analogue in human organisms; thus a chemical compound that will act specifically on the parasite without harming the host would be an ideal remedy.

In his search for such potent chemotherapeutic compounds Ehrlich was influenced by two guiding concepts: (1) that of a drug that in a single dose would destroy all the parasites, and (2) that of the chemotherapeutic index. The latter concept was based on the postulate that no alien chemical compound can be completely harmless to the human organism but that it will be the more acceptable the higher the dose of the drug tolerated by the organism of the host in relation to the lowest dose sufficient to eliminate the pathogen

The concept of selective action of chemotherapeutic compounds must therefore be considered from both the qualitative and the quantitative aspect. The available antimalarial drugs have a well defined range of action against different species of malaria parasites of animals and man. They also have a varying degree of activity against the different stages of development of the plasmodia in the vertebrate host and in the invertebrate vector. Thus any assessment of the value of an antimalarial compound must be based on an understanding of the life cycle of the plasmodia and of the activity of groups of compounds in relation to the various stages of this life cycle.

The efficacy of an antimalarial drug depends not only on its specific action on a given species and strain of a malaria parasite but also on such host-related factors as the speed of absorption, the degree of concentration in the plasma and in the erythrocytes, the localization in the tissues, and the rate of degradation and excretion. The response of the human host is also modified by any previous encounter he may have had with the malaria parasite, which has a bearing on his immune status.

Thus the triad of the drug, the parasite and the host must be considered.

Malaria parasites and the course of infection

Human malaria parasites belong to the zoological order of Haemosporidia, family Plasmodiidae, genus *Plasmodium*. They have the following characteristics defined by Garnham (1966): they undergo one cycle of asexual division in the tissues (*exoerythrocytic schizogony*) and another cycle of pigment-producing, asexual division in the red blood cells (*erythrocytic schizogony*) of the vetebrate host; they also undergo a sporogonic development (*sporogony*) in the body of the mosquito.

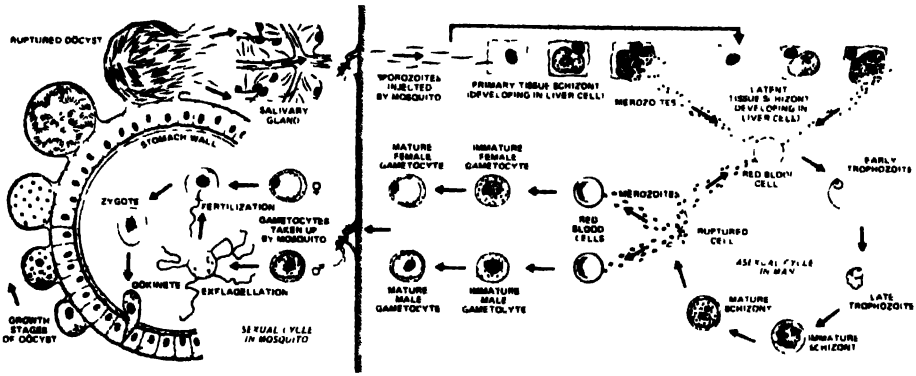
The genus *Plasmodium* can be further divided into two subgenera: *Plasmodium* in the strict sense and *Laverania*. The latter subgenus includes *P. falciparum*, the exoerythrocytic schizonts of which occur for one generation only; the gametocytes of this species are crescentic. The subgenus *Plasmodium* comprises *P. vivax*, *P. ovale* and *P. malariae*, the respective agents of benign tertian, ovale tertian and quartan malaria.

The development of each of the four species of human plasmodia starts with the phase in which the direct progeny of sporozoites injected into the circulation by the bite of an infected anopheles enter the liver, where they undergo growth and multiplication in the parenchymatous cells. This *pre-erythrocytic tissue schizogony* is completed towards the end of the incubation period of the infection, when large numbers of *tissue merozoites* from ruptured tissue schizonts are released into the blood stream. In this form the parasite invades the erythrocytes, grows and multiplies cyclically from trophozoites to mature blood schizonts according to the species of the parasite, and produces all the clinical symptoms of the disease. Some erythrocytic forms develop into two types of sexual parasites (*gametocytes*), which will unite when taken up by a suitable female anopheles that has ingested the blood of the infected individual. Eventually, after the gradual stages of *ookinete* and *oocyst*, large numbers of sporozoites are produced and stored in the salivary glands of the anopheles; they ensure the transmission of the disease when injected into a new human host (Fig. 2).

The above cycle of development occurs in all four species of human malaria parasites and terminates at this point in *P. falciparum*. It was held that in some species the merozoites originating from pre-erythrocytic tissue schizogony reenter liver cells and continue their development as secondary exoerythrocytic forms which are responsible for producing relapses of malaria with clinical symptoms.

This hypothesis of a cyclical secondary maturation and reinvasion of tissue schizonts has now been strongly challenged and there is more evidence of a latent tissue stage (hypnozoites) in the hepatic cells for *P. vivax* and *P. ovale*. With regard to *P. malariae*, some recent evidence indicates that there is no secondary exoerythrocytic schizogony in this species of *Plasmodium* and that "relapses" of *P. malariae* infection may originate from erythrocytic forms remaining in the body for a considerable time.

Fig. 2 Cycles of development of malaria parasites in the anopheline mosquito and in man. Action of various groups of antimalarial drugs on different parts of each cycle[1]



From World Health Organization (1963) *Terminology of malaria and of malaria eradication* Geneva

Some infections with *P. vivax* seen predominantly in northern and north-eastern Europe may have protracted incubation periods of 8–10 months' duration. Whether such long incubations are attributable to very small numbers of injected sporozoites or to an intrinsic character of the plasmodium remains controversial. On the basis f the latter hypothesis some authors gave to this type of plasmodium the tentative subspecific name of *P. vivax hibernans*. However, recent studies suggest that *P. vivax* is a polymorphic species with two types of sporozoites (tachysporozoites and bradysporozoites) producing short incubation periods or long incubation periods depending on the numerical proportion of the two types transmitted by the mosquito.

The duration of the pre-erythrocytic stage, which influences the length of the incubation period, is usually short in *P. falciparum* (5.5–7 days) and *P. vivax* (6–8 days), somewhat longer in *P. ovale* (9 days), and longest (13–16 days) in *P. malariae*. These differences have a bearing on the therapeutic approach to malaria. Moreover, the four species also differ in the number of tissue merozoites released by the hepatic schizonts after maturation: *P. falciparum*—about 40 000 from each schizont, *P. ovale*—about 15 000, *P. vivax*—over 10 000, and *P. malariae*—2000. The large number of merozoites released by *P. falciparum* and the short incubation period explain the high levels of parasitaemia and severe symptoms often seen in this infection.

The conventional term *incubation period* covers the time between the day of the infection and the appearance of clinical symptoms (premonitory signs or a febrile attack). This should be distinguished from the *prepatent period*, which is the time that elapses between the infection and the first appearance of

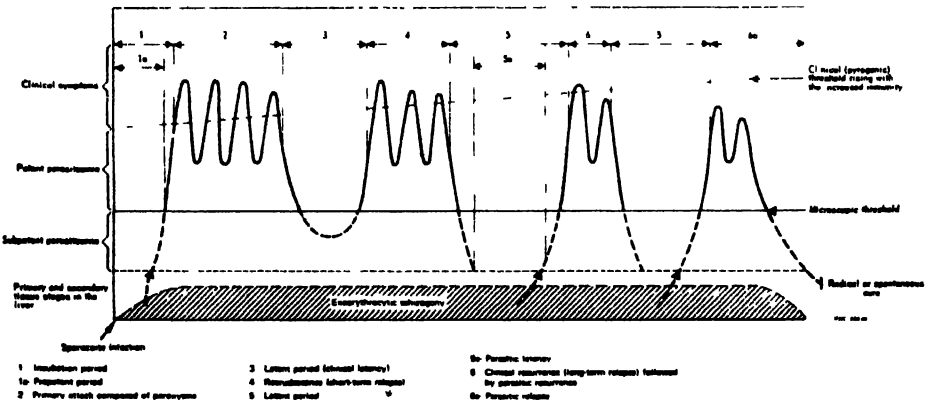
plasmodia in the peripheral blood. There is also a *subpatent period*, when parasites may be present in the blood in such small numbers that they cannot be detected by ordinary microscopical examination of one or several blood films.

These stages are followed by clinical symptoms, usually associated with patent parasitaemia. The primary attack is composed of a number of *paroxysms* and, if not cured at this stage, is usually succeeded by a period in which the course of the infection is influenced by the host's immune response.

The occurrence of a paroxysm depends on the timing of the schizogony of a generation of erythrocytic parasites (hence the terms "tertian" or "quartan" malaria). It seems that there is also a definite quantum of parasites the presence of which in the blood will cause febrile symptoms. This level varies greatly in different subjects according to their degree of immunity.

The primary attack is frequently followed at intervals by other attacks caused by the same original infection. These subsequent attacks (relapses) have been variously designated by different authors in relation to the time of their occurrence as *recrudescences* (short-term relapses due to the survival of erythrocytic forms) or as *recurrences* (long-term relapses). The origin of relapses of *P. vivax* malaria has recently been related to the delayed formation of tissue schizonts from latent "hypnozoites" produced by some sporozoites (see Fig. 2). The relapse patterns, the presence or absence of symptoms with relapses, and the duration of the incubation period at primary infection have been used to classify *P. vivax* infections into three or more types, each with a particular geographical distribution. Symptomless relapses are called *parasitic relapses*, and the periods between relapses are often known as periods of *latency* (Fig. 3).

Fig. 3 Diagram of the course of a malaria infection showing the difference between recrudescences and recurrences[1]



[1] From: World Health Organization (1963) *Terminology of malaria and of malaria eradication*. Geneva

The longevity of untreated or partially treated natural malaria infection in man has a bearing on both its prevention and its treatment. It is generally admitted that the duration of *P. falciparum* infection seldom exceeds one year and that *P. vivax* (and presumably *P. ovale*) infections usually die out within 3–4 years. On the other hand, infections with *P. malariae* may persist for many years. Cases have been described of "relapses" of quartan malaria occurring 30–40 years after the original infection. Thus the time-based criteria for the radical cure of quartan malaria must be assessed with caution.

The Role of Immunity in the Chemotherapy of Malaria

Malaria immunity may be defined as the capacity of resisting the infection brought about by all the processes involved in destroying the plasmodia or in limiting their multiplication. It also comprises the factors that modify the effects of the invasion of the organism by malaria parasites and aid in the repair of damaged tissues.

There are two types of immunity, natural and acquired. *Natural immunity* to malaria is an inherent property of the host, a refractory state or an immediate inhibitory response to the introduction of the parasite, not dependent on any previous infection with it. An example of such a state is the natural resistance of man to an infection with avian or rodent plasmodia.

There are several genetic aspects of resistance to some types of malaria infection. Thus the partial insusceptibility of black ethnic groups to infection with *P. vivax* is apparently associated with the absence in these populations of the Duffy red blood cell determinants that are common in other ethnic groups.

The high incidence of the abnormal haemoglobin S (HbS or sickle-cell haemoglobin) in many parts of the world, but particularly in Africa, was difficult to explain since this genetic defect is eventually lethal in its homologous expression (SS) as in sickle-cell anaemia, though apparently relatively harmless in its heterozygous expression (AS), which results in the sickling of erythrocytes when the oxygen tension is low. The similarity of the geographical distribution of haemoglobin S (HbS) and of holoendemic falciparum malaria is striking and it has been suggested that the maintenance of the high frequency of HbS in the population might be due to the selective advantage that the heterozygote conferred over the adverse effects of falciparum malaria. This hypothesis is now generally accepted. The mechanism whereby sickle-cell haemoglobin partially protects its bearer from the severe effects of falciparum malaria is not fully understood, but it appears that the infected erythrocyte, which shows a tendency to sickling when the oxygen tension is lowered, is disposed of faster by the macrophages and other cells of the reticulo-endothelial system. Recent studies indicate that haemoglobin S has a detrimental effect on the proliferation of *P. falciparum*, affecting both parasite invasion of the red blood cell and growth inside it. Actual sickling of the cells concerned is not necessary.

There is no clear evidence that other genetic variants of haemoglobin such as HbC, HbF (fetal) or HbE confer protection against falciparum malaria.

It appears that genetic deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD) also exerts a protective effect against severe infection with *P. falciparum*. This red cell enzyme defect appears to be harmless unless the red cells are challenged in some way, usually by exposure to various drugs (including sulfonamides and primaquine). G6PD deficiency is inherited as a sex-linked trait with full expression in males. There are many variants of this enzymopathy. The evidence for the protective effect of G6PD deficiency against malaria is not as strong as for haemoglobin S.

Acquired immunity may be either active or passive. *Active immunity* is an enhancement of the defence mechanism of the host as a result of a previous encounter with the pathogen. *Passive immunity* is conferred by the prenatal or postnatal transfer of protective substances from mother to child or by the injection of such substances contained in the serum of immune persons. There is good evidence for such *congenital (or neonatal) immunity* in newborn babies of highly immune mothers in endemic malarious areas of the world.

The protection acquired by a host against subsequent reinfection with the homologous strain of the relevant species of the malaria parasite is maintained for a variable period of time, depending on the degree and duration of the immune response to the antigen. There is little if any protective effect of such immunity against a species of plasmodium different from that which caused the initial infection.

In highly endemic areas where transmission of malaria continues throughout the greater part of the year, the population develops and maintains a high degree of immune response while at the same time there is a nearly permanent presence of very small numbers of malaria parasites in many subjects, mainly adult. This state of resistance in a previously infected host coupled with asymptomatic parasitaemia is known as *premunition*. Such a state of collective immunity is acquired slowly, and infants and young children may suffer severely from malaria and many die. Those, however, who survive to adulthood show little evidence of the adverse effects of the attenuated infection.

Malaria immunity develops following invasion of the organism by the erythrocytic forms of malaria parasites;[1] there is no convincing evidence that the exoerythrocytic stages have any significant effect on the immune response. There is no cellular response to these stages of the parasite in the liver.

The physical basis of malaria immunity depends on the activity of both *humoral* and *cellular factors*, though the physiological conditions of the host also play some, albeit a little known, part.

[1] The influence of acquired immunity on the course of malaria infection is indicated in Fig. 3 by the rising clinical threshold.

The humoral factors are represented by the *antibodies* that appear in the blood. They comprise opsonins, precipitins and agglutinins; the most important protective antibodies are carried on the gammaglobulin components (IgG) of the serum. The cellular factors are macrophages and other cells produced by the reticuloendothelial (lymphoid-macrophage) system of the spleen, liver and bone marrow, which undergoes intense proliferation following malaria infection. The phagocytic activity of these cells, which dispose of a large number of parasites, has been well recognized as one of the main mechanisms of defence. The interdependence of the cellular and humoral factors has recently become better known.

Today the tendency is to view the immune response as an integrated phenomenon involving the T lymphocytes and B lymphocytes. T lymphocytes, which comprise the bulk of circulating lymphocytes, give rise to lymphoblasts, which can be cytotoxic to plasmodia that they recognize. These lymphoblasts do not synthesize antibody but stimulate B lymphocytes and the derived plasma cells to antibody formation. The recognition of antigen, which precedes antibody production, is also assisted by macrophages and by the action of opsonins.

A number of soluble antigen fractions in falciparum infections have been identified and classified as L (labile), R (resistant) and S (stable) on the basis of their susceptibility to heat.

Malaria antibodies have been detected in the sera of infected persons by the indirect fluorescent antibody test, immunoprecipitation techniques and other serological tests. Antibody activity has been found in IgG, IgM and IgA immunoglobulin fractions of immune sera, but predominantly in the IgG fraction. The factors that determine the Ig class of antibodies synthesized by B cells in response to malaria antigens are not known.

Studies made in nonimmune volunteers have shown that serum concentrations of IgG, IgA and IgM rise shortly after the beginning of parasitaemia. IgG and IgM increase more than IgA, while IgG persists longer.

Although the specific protective antiplasmodial factor forms only a small part of IgG, it can be transmitted passively by the injection of a large amount of the IgG fraction of immune human serum. Moreover, in sera from African newborn children who possess some transient resistance to malaria infection IgG, transmitted from the immune mother across the placenta, is present in considerable amounts.

The working of protective immunity to malaria is seen best in holoendemic areas of tropical Africa. Although infection of the placenta is frequent, congenital malaria is rare because of the prenatal transfer of specific IgG antibodies from the maternal blood across the placenta. Such passively acquired immunity is transient, and after the first 4–6 months of life many young children have a high parasitaemia and severe clinical illness leading to a high fatality rate. In older children clinical illness is less common, but enlarged spleens and parasitaemia are common; in adults parasitaemia becomes

infrequent and of low density, clinical illness is rare, and palpable splenic enlargement is uncommon

Antibodies found in high prevalence and titre in the sera of babies born from highly immune mothers decay in the early months of life and their titre becomes low By the third year, in response to infection, the prevalence of parasitaemia may reach 100 %. The antibody titre rises slowly to a peak by early adulthood and subsequently remains at a high level Such age-related antibody profiles in various populations form a valuable method for the assessment of malaria endemicity and for evaluation of the efficacy of malaria control activities

The immunity factors in malaria are directed mainly against the erythrocytic forms of plasmodia, while the exoerythrocytic stages in the liver do not appear to be affected by either the humoral or the cellular response of the defence mechanism Because of the protective effect of immunity to malaria acquired in the course of previous infections it is commonly found that antimalarial drugs in individuals previously exposed to malaria have a more pronounced preventive and curative action than in nonimmune subjects However, the degree of naturally acquired immunity cannot be adequately measured, though it is usually related to the duration of exposure, i e , to the age of the person concerned in highly endemic areas Acquired immunity is most effective against infection with the same species and strain of the parasite, though to some degree it operates against other species and strains of plasmodia

Biochemistry of Malaria Parasites and Evaluation of Antimalarial Action

Study of the biochemical requirements of human plasmodia meets with many difficulties because of the obligatory intracellular life of malaria parasites Nevertheless, work on the plasmodia of birds, rodents and monkeys has provided much information that could be and has been extended to malaria parasites of man A great amount of information has been gathered on the nature and distribution of various substances in the life stages of plasmodia and on their nutritional requirements and metabolic pathways The relationship of these studies to the development of chemotherapeutic products is too obvious to need any emphasis

The main reactions involved in plasmodial metabolism are (1) phosphorylation of glucose, which provides the energy required, (2) oxidative processes, which are maintained by the oxyhaemoglobin of the host cell, (3) enzyme breakdown of the globin portion of haemoglobin into amino acids and peptides and (4) synthesis of lipids Among inorganic substances investigation of the uptake and utilization of phosphorus by plasmodia has received particular attention because of the role of this element in the formation of the nucleic acids, DNA and RNA The part played by

carbohydrates in respiration has been elucidated for some plasmodial species. It appears that a number of enzymes catalyse the key reaction of the glycolytic series of the Embden-Meyerhoff-Parnas scheme, converting glucose to lactate. Evidence has been obtained in several avian species of the operation of the tricarboxylic acid cycle (Krebs cycle) in the aerobic metabolism of carbohydrates, though some mammalian plasmodia do not possess the relevant enzymes. Evidence concerning pentose phosphate pathways in aerobic glycolysis has been controversial. Information on the functional cytochrome oxydase system is incomplete, although it appears to exist in *P. falciparum*.

Typical protozoan mitochondria are present in plasmodia and are the probable site of action of enzymes. Mitochondria supply the parasites with the energy that they require, particularly during their extracellular existence.

As for protein metabolism, it appears that the main source of amino acids utilized by malaria parasites is red cell haemoglobin. The first step is cleavage, which liberates amino acids and haemozoin (malaria pigment). Among the free amino acids in the serum, L-methionine and L-isoleucine are essential for the growth of mammalian plasmodia. Several other amino acids are also utilized and tetrahydrofolic acid is a co-factor in this process, so that the use of antifolates as antimalarials may block this and other folic-acid-dependent reactions (e.g. DNA synthesis). Nucleic acid metabolism in plasmodia is similar to that of other organisms and both DNA and RNA are synthesized during nuclear development and division. All the enzymes necessary for the biosynthesis of folate co-factors have been found in several species of plasmodia. It has been established that two types of antimalarials, the sulfonamides and the antifolates, affect through their mechanism of action the synthesis of folate co-factors by the enzymes of the parasite; the sulfonamides inhibit dihydropteroate synthesis and the antifolates, such as pyrimethamine, bind plasmodial dihydrofolate reductase.

Chloroquine and other 4-aminoquinolines interact with nucleoproteins of the parasite, but this does not appear to be their primary mode of action. Primaquine and its congeners appear to act through interference with mitochondrial respiratory processes.

The plasmodial metabolism of lipids is not fully known. Although there is an increase of the lipid content in parasitized erythrocytes, there is no clear evidence of phospholipid synthesis in human plasmodia, though the role of stearic acid has been studied.

Present knowledge of biochemical changes during growth in malaria parasites has been obtained chiefly through the cultivation of erythrocytic forms of animal plasmodia *in vitro*. Recent successes in maintaining long-term *in vitro* cultures of *P. falciparum* are bound to open new vistas in the understanding of the various aspects of the metabolism of this and other species, and so improve the prospects of chemotherapy.

It is obvious that understanding of the metabolic processes of plasmodia may offer much guidance in the evaluation of candidate antimalarial

compounds. Most of the early test procedures were carried out on *P. relictum* and *P. cathemerium* of canaries; later *P. lophurae* and *P. gallinaceum* of ducklings and chicks were widely used. The discovery of *P. berghei* opened up the possibility of tests in rodents. For further evaluation of drugs and their physiological disposition in animals closer to man various species of simian malaria parasites were used, though *P. cynomolgi* and *P. knowlesi* of rhesus monkeys (*Macaca mulatta*) were of particular value for the study of the chemotherapeutic effects on both blood and tissue stages. More recently both normal and drug-resistant strains of *P. vivax* and *P. falciparum* have been adapted to owl monkeys (*Aotus trivirgatus*) for drug evaluation.

Promising antimalarial compounds undergo tests for acute and chronic toxicity by various routes of administration so as to show the effects on the most vulnerable organs. The final evaluation of promising and least harmful potential antimalarials is carried out either on volunteers with induced malaria or on naturally infected subjects in hospitals or in the field.

Biological Classification of Antimalarials

Since various stages in the life cycle of malaria parasites show different susceptibility to antimalarial drugs, the latter may be classified into the following groups, as shown in Fig. 4 and Table 1:

1. *Tissue schizontocides* (used for causal prophylaxis) acting on the pre-erythrocytic stages of the parasite (primary tissue forms or primary exo-erythrocytic forms) and thus completely preventing invasion of the red blood cells.

Fig 4 Simplified diagram of action of antimalarial compounds on different parts of the cycle of development of *P vivax* and *P ovale* in the mosquito and in man

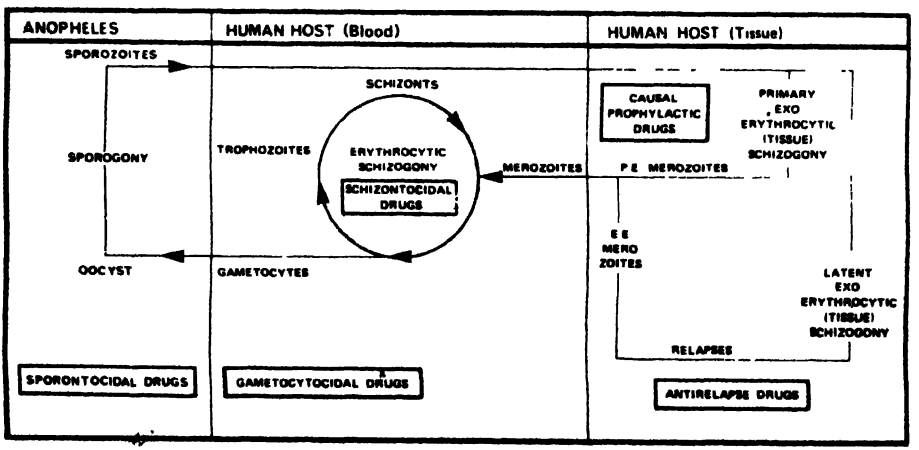


TABLE 1 ACTION OF COMMONLY USED DRUGS ON THE CYCLE OF DEVELOPMENT OF MALARIA PARASITES

Drug	Sporozoites	Tissue phase during the incubation period	Erythrocytic phase		Latent tissue phase (responsible for elapses)	Development of gametocytes in the mosquito (sporontocidal action)	Chemical class of the relevant antimalarial compound
			Asexual parasites	Sexual forms (gametocytes)			
Quinine	No action	No action	Fast action	Active against *P vivax* and *P malariae* No direct action on *P falciparum*	No action	No action	Cinchona alkaloids
Mepacrine	No action	No action	Fast action	As quinine	No action	No action	9 aminoacridines
Chloroquine Amodiaquine	No action	No action	Fast action	As quinine	No action	No action	4 aminoquinolines
Primaquine	No action	Active but not used for prophylaxis	Active but only in toxic doses	Direct and fast action on all species but particularly *P falciparum*	Highly active	Highly active	8 aminoquinolines
Proguanil	No action	Active, particularly on *P falciparum*	Active but relatively slow	No direct action	No action	Highly active	Biguanides
Pyrimethamine	No action	Probably as proguanil	As proguanil	No evidence of direct action	Some action on *P vivax*	Little evidence	Diaminopyrimidines
Sulfones and Sulfonamides	No action	Possible action	Moderate action when given alone	As pyrimethamine	Little evidence		Sulfones and sulfonamides comprise a large number of short acting and long acting compounds
Mefloquine	Probably no action	Probably no action	Marked action	As quinine	Probably no action	Probably no action	Quinolinemethanols

2. *Tissue schizontocides* (used as antirelapse drugs) acting on the exoerythrocytic stages or tissue forms of *P. vivax* and *P. ovale* and thus able to achieve radical cure of these infections [2]

3. *Schizontocides* (blood schizontocides or schizontocidal drugs) acting on the erythrocytic stages of parasites commonly associated with acute disease, though these stages may be present in some infections accompanied by few clinical symptoms. Schizontocides may achieve clinical cure or suppression to a subpatent level of infection with all four species of malaria parasite susceptible to their action. They also act on the sexual erythrocytic forms of *P. vivax*, *P. ovale* and *P. malariae* but not directly on the mature gametocytes of *P. falciparum*.

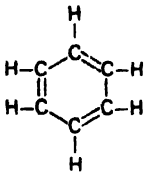
4 *Gametocytocides* (gametocytocidal drugs) destroying all sexual forms including those of *P. falciparum*, they also act on the developmental stages of malaria parasites in the anophelines and some of them form the next group of drugs.

5. *Sporontocides* (sporontocidal drugs) preventing or inhibiting the formation of oocysts and sporozoites in anophelines that have fed on carriers of gametocytes. They interfere with the transmission of malaria, though they may have no direct action on gametocytes in the human host. These drugs have also been called antisporogonic drugs Writers in Russian refer in this context to the "gamostatic" and "gamotropic" effects of drugs

General Chemical Structure of Antimalarials

The benzene ring occurs in the molecular structure of all the classical antimalarials. The benzene molecule contains 6 carbon atoms and 6 hydrogen atoms (i.e., its molecular formula is C_6H_6); the carbon atoms are arranged in the form of a flat regular hexagon, a hydrogen atom (lying in the same plane as the hexagon) being attached to each of them. This molecule is conventionally represented by the structural formula proposed by Kekulé in 1865 (I), although the simplified form II is often used for convenience. In fact, however, this representation is incorrect, and the structure of benzene has long been an enigma. To account for the observed properties of benzene, Kekulé suggested in 1872 that the molecule underwent oscillation, the single and double bonds constantly changing position "back and forth" between forms II and III. This was later shown to be incorrect, and today it is recognized that the benzene molecule cannot be adequately represented by any single structural formula. Instead, it is thought of as being a "hybrid" of several different structures (IV–VIII). The double-headed arrows in this representation do not indicate that a reversible reaction, an interchange, or any other dynamic process takes place: they mean only that the benzene

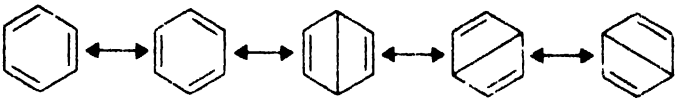
[2] There is now some evidence, both circumstantial and experimental, that *P malariae* has no latent exoerythrocytic forms



I

II

III

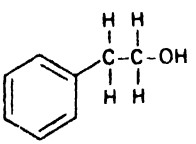


IV V VI VII VIII

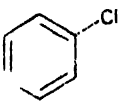
molecule is best represented by considering together *all* the structures to which they point. Technically, benzene is said to be a "resonance hybrid" of these different structures. This term is unfortunate, since it implies the occurrence of oscillation of single and double bonds as postulated by Kekulé, which does not in fact occur. The existence of single and double bonds in the benzene ring would necessitate the localization of electrons, but the electrons involved are in fact completely delocalized; "resonance" means *electron delocalization*. As a consequence, the benzene ring does not contain 3 single bonds and 3 double bonds, but, rather, 6 bonds that are intermediate between single and double bonds. To overcome the difficulty of representation, the benzene ring is often drawn as a hexagon enclosing a circle (IX); however, the conventional hexagon II is widely used, and is employed in this text.

Other atoms can be substituted for any of the hydrogen atoms in the benzene molecule. A chain of 2 or more atoms (usually carbon atoms, to which other atoms may in turn be attached) attached to one of the carbon atoms of the "skeleton" or ring of a cyclic compound is known as a *side chain* (X).

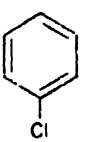
IX



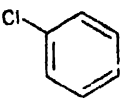
X



XI



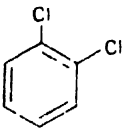
XII



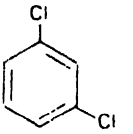
XIII

Since all the carbon atoms in the benzene ring are equivalent, the position of a single substituent is immaterial. For example, the 3 structures XI, XII, and XIII are identical, and all represent chlorobenzene. However, when 2 or more substituents are present, their relative positions on the ring are of great importance. For example, structures XIV, XV, and XVI represent com-

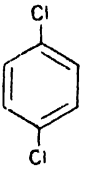
pounds having the same molecular formula, $C_6H_4Cl_2$, but different spatial arrangements of the chlorine substituent. Such compounds, which are called *isomers*, may have similar chemical properties but different physical properties. Since they are different compounds, a distinctive name for each is necessary.



XIV



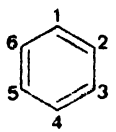
XV



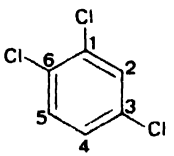
XVI

The position of each substituent on a ring is indicated by means of a *locant* (usually a number) preceding the name of the substituent and indicating the carbon atom to which it is attached. If there are 2 or more identical substituents a *multiplicative prefix* (di, tri, tetra, etc., according to the number of substituents) is added to the name of the substituent. The entire expression thus obtained is added to the name of the ring system. For example, the names of structures XIV, XV, and XVI are 1,2-dichlorobenzene, 1,3-dichlorobenzene, and 1,4-dichlorobenzene, respectively. Substitution in these 3 positions on the benzene ring is sometimes referred to as *ortho-* (on adjacent carbon atoms), *meta-* (on next-but-one carbon atoms), and *para-* (on "opposite" carbon atoms) substitution.

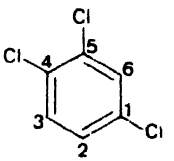
The assignment of locants is a matter of some importance. Since all the carbon atoms in the benzene ring are equivalent, numbers cannot arbitrarily be assigned to them as in XVII. Instead, locants are assigned *in each individual case* in such a way as to produce the lowest possible set of numbers. For example, XVIII, XIX, and XX show 3 of the possible ways of numbering the carbon atoms in the benzene ring of the compound represented. These give, for the positions of the substituents, the numbers 1,3,6-, 1,4,5-, and 1,2,4-, respectively. Of these, the lowest set is 1,2,4-, and the compound is therefore named 1,2,4-trichlorobenzene.



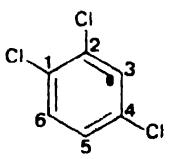
XVII



XVIII



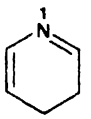
XIX



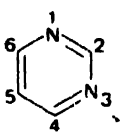
XX

Atoms other than carbon, called *heteroatoms*, may replace one or more of the carbon atoms of the benzene ring, the product being called a *heterocycle*.

The heteroatom of importance in the chemistry of antimalarials is nitrogen. If a single nitrogen atom replaces one of the carbon atoms of the benzene ring, the product is pyridine, the basis of a number of antimalarial compounds. When such a replacement is made, the situation with respect to numbering of the ring atoms changes the nitrogen atom being assigned the number 1 and this number never varying However, pyridine is a resonance hybrid; consequently, the carbon atoms in the ring may be numbered either clockwise or counter-clockwise, the direction that is taken in any given case being determined by the requirement that locant numbers be as low as possible. For example, if a chlorine atom is attached to 1 of the 2 carbon atoms adjacent to the nitrogen atom in the ring, it makes no difference whether the "left" or the "right" carbon atom is involved· in each case the product is the same and is named 2-chloropyridine



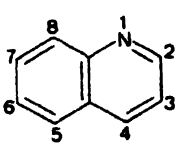
Pyridine



Pyrimidine

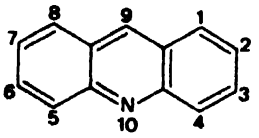
If a second nitrogen atom is introduced into the pyridine ring, replacing the carbon atom that is "next but one" to the existing nitrogen atom, the product is pyrimidine Following the "lowest possible numbers" rule, the 2 heteroatoms in the pyrimidine ring are assigned the numbers 1 and 3, with the result that the numbering of the pyrimidine-ring atoms is fixed Substitution of amino ($-NH_2$) groups at the 2 and 4 positions of the pyrimidine ring gives 2,4-diaminopyrimidine, the basis of pyrimethamine and other antimalarial compounds.

Ring systems may be joined in such a way that 2 carbon atoms are common to 2 or more rings This process is known as ring *fusion*, and the resulting compound is said to be polycyclic Fusion of a benzene ring and a pyridine ring in such a way that the 2 and 3 atoms of the latter are common to both rings gives quinoline Substitution of a complex group at the 4 position of quinoline gives 2 of the important cinchona alkaloids, and additional substitution at the 6 position gives the other 2 important members of this group of alkaloids. Quinoline also is the basis of a number of synthetic antimalarial compounds Substitution of an amino group at the 4 or the 8 position of quinoline gives 4-aminoquinoline and 8-aminoquinoline respectively, each of which is the basis of an important group of antimalarials.

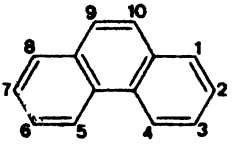


Quinoline

Fusion of 2 benzene rings and a pyridine ring at the 2,3 and 5,6 positions of the latter produces acridine (the different numbering of this structure should be noted). Substitution of an amino group at the 9 position of the acridine ring gives 9-aminoacridine, the basis of another group of antimalarials.



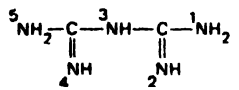
Acridine

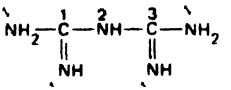


Phenanthrene

Phenanthrene, another polycyclic compound on which a number of antimalarials are based, is produced by the fusion of 3 benzene rings.

Finally, there are 3 groups of antimalarials that are based on noncyclic structures. These are the biguanides, the sulfones, and the sulfonamides. Two different systems are in use for assigning locants to the biguanide structure, as

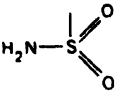




Biguanide

shown. In one, only numbers are used; the other makes use of a combination of numbers and italic capital letters with "primes" (*N'*, etc.). Sulfones and sulfonamides contain the groups shown. All antimalarial biguanides, sulfones, and sulfonamides have cyclic substituents that contain the benzene ring.

Sulfone



Sulfonamide

Discussion of Specific Antimalarials

The existing antimalarial drugs are described below in the following groups, in relation to their chemical structure and their biological activity.

1. Cinchona alkaloids (e.g. quinine)
2. 8-aminoquinolines (e.g. primaquine, quinocide)

3. 9-aminoacridines (e.g. mepacrine)
4. 4-aminoquinolines (e.g. chloroquine, amodiaquine)
5. Biguanides (e.g. proguanil, chlorproguanil)
6. Diaminopyrimidines (e.g. pyrimethamine, trimethoprim)
7. Sulfones and sulfonamides
8. Quinolinemethanols and phenanthrenemethanols
9. Antibiotics
10. Other compounds.

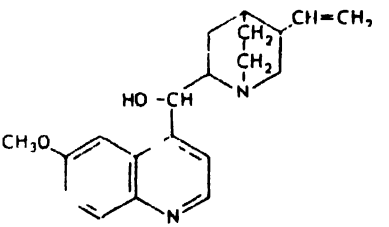
Cinchona alkaloids

The bark of cinchona trees contains a mixture of some 10 alkaloids, but most of them are not crystallizable and are referred to collectively as "quinoidine", a term applicable to the residue after the removal of the 4 valuable alkaloids quinine, quinidine, cinchonine and cinchonidine. The first 2 alkaloids have a higher potency against human malaria parasites than against avian plasmodia; hence the need for caution in interpreting the results of screening tests in only one animal test model.

The mixtures of alkaloids derived from cinchona bark were known as cinchona febrifuge and totaquina. The latter mixture, introduced in the 1930s by the Health Organisation of the League of Nations, had a standard composition of 15 % of quinine and other crystalline alkaloids.

Quinine, the most important compound of this group, has a complex chemical structure composed of the quinoline ring (with a methoxy group at the 6 position), the quinuclidine complex ring with an attached vinyl group and the connecting link in the form of a hydroxylated methylene group.

Any alteration of the chemical structure of quinine changes the pharmacological action of the compound.



Quinine
6-methoxy-α-
(5-vinyl-2-quinuclidinyl)-
4-quinoline methanol

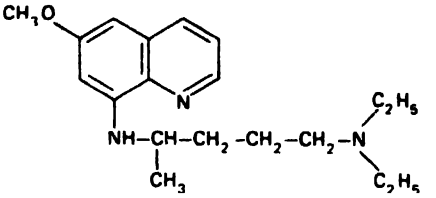
Although the correct structure of quinine had already been suggested in 1908, synthesis of this compound was achieved by Woodward & Doering only in 1944. However, the difficulty of synthesis was such that this is not a commercially viable method. The need for a supply of reasonably priced synthetic quinine led chemists to explore less complex synthetic methods and there may be some promise of achieving this goal.

A variety of organic salts of quinine have been prepared, but they have not been either more potent than quinine or more long-term in their action.

8-aminoquinolines

The search for synthetic antimalarials pioneered in Germany by Schulemann and his colleagues had its first success in the 1920s, with the replacement of one of the methyl groups of methylene blue by the dialkylaminoalkyl side chain. The demonstration that this had a high activity on avian malaria led to the discovery of *pamaquine* (Plasmochin).

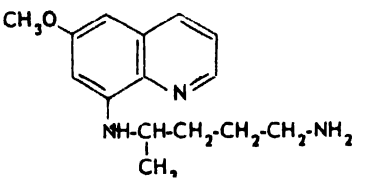
Pamaquine
8[(4-(diethylamino)-1-methylbutyl]amino-6-methoxyquinoline



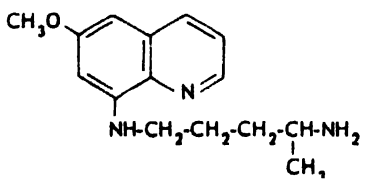
In this first synthetic antimalarial the 6-methoxyquinoline is combined with the basic side chain. The original German studies have never been published in full and the initial structure-activity correlations are attributable to studies by Fourneau in France, Robinson in England and Magidson in Russia. Although the 6-methoxy substituent on the quinoline nucleus is not essential for high activity, all of the clinically useful 8-aminoquinolines (pamaquine, rhodoquine, pentaquine, isopentaquine, primaquine and quinocide) contain it. Only *primaquine* is now widely used, mainly as a gametocytocidal and antirelapse drug. *Quinocide*, synthesized in the USSR in 1952, differs from primaquine only in the position of the methane molecule in the alkylamino chain and has very similar therapeutic properties and side effects, though its chemotherapeutic index is somewhat lower.

Primaquine and quinocide have the following structural formulae:

Primaquine
6-methoxy-8-(4'-amino-1'-methylbutylamino)quinoline



Quinocide
6-methoxy-8-(4'-amino-4'-methylbutylamino)quinoline



Both compounds may produce adverse side effects such as epigastric pain and haemoglobinaemia and this, together with the need for frequent dosage, limits their value as prophylactic, i.e., sporontocidal agents. Nevertheless, the 8-aminoquinolines are a fertile field for the development of new drugs.

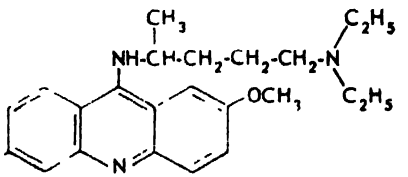
A combination of two 8-aminoquinolines, pamaquine (Praequine) and rhodoquine [6-methoxy-8-(3-diethylamino-n-propylamino) quinoline], known as 710 F, has been quite widely used in France under the name Rhodopraequine.

9-aminoacridines

When it was realized that pamaquine was not a substitute for quinine further research continued. It was the introduction of the basic di-alkylaminoalkyl chain into the acridine nucleus that resulted in the discovery of *mepacrine* (Atebrin) by Kikuth and his colleagues in 1932 They replaced the quinoline ring of pamaquine by acridine, a yellow dye, in the hope of decreasing the toxicity and prolonging the action of the new compound, which was the best of some 12 000 compounds tested by German scientists between the two World Wars.

Although the quinoline ring of pamaquine had been expanded to form acridine, the aminoalkylamino side chain of pamaquine was found to be essential for activity; it is situated in the 9 position opposite the nitrogen atom, as in quinine. The presence of a methoxy group and of a chlorine atom endowed the compound with other desirable properties. Because the hydrogen atom can assume 2 alternative positions in the molecule, mepacrine has 2 structurally isomeric forms which exist in a dynamic equilibrium.

Mepacrine
2-methoxy-6-chloro-9-
(4'-diethylamino-1 -methylbutylamino)acridine



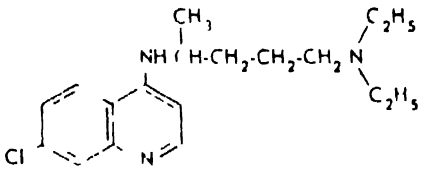
Many similar acridine derivatives (acroquine, aminoacrichin, azacrin) were developed in the United Kingdom and the USSR but failed to prove superior to mepacrine. During the Second World War mepacrine became the chief substitute for quinine. While it was of unquestionable value during the 1940s, when quinine was not available, it is today obsolete, wholly replaced by other compounds.

4-aminoquinolines

The presence of the quinoline ring in the structure of quinine and mepacrine as well as the known therapeutic properties of 8-aminoquinolines

were the logical precursors of further studies on antimalarials. Several compounds were investigated by German workers, especially Schönhofer, and those with the basic dialkylaminoalkyl side chain in the 4 position showed some promise. Eventually, two of them, chloroquine (Resochin) and sontoquine (Sontochin) were selected for further trials in North Africa, the second being given preference by the Germans on the ground that it was less toxic. Supplies of this drug together with German research data fell into the hands of the United States forces, thanks to the cooperation of two French scientists, Décourt and Schneider. This stimulated further studies in the USA, where these compounds and a number of other 4-aminoquinolines were evaluated on volunteers. *Chloroquine* (Aralen) proved to be the most effective among them and less toxic than any other.

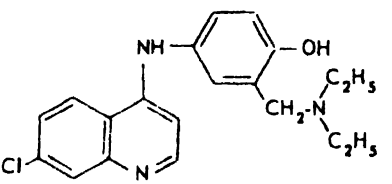
The structural formula of chloroquine is as follows:



Chloroquine
7-chloro-4-(4-diethylamino 1'-methylbutylamino)quinoline

It contains the same alkyl chain as mepacrine but differs from the latter in having a quinoline instead of an acridine nucleus and in lacking a methoxy radical. All of the clinically useful drugs of this series have the chloro substituent in position 7 and this seems to be connected with their specific antiplasmodial action.

Amodiaquine, another member of this group, has a structural formula in which the alkylamino side chain is replaced by an anilino group. Its manner of antimalarial action is equal to that of chloroquine, but amodiaquine appears to be marginally more active than chloroquine on strains of *P. falciparum* resistant to this drug. Amodiaquine base is less bitter than its salts and this is of interest in paediatric practice

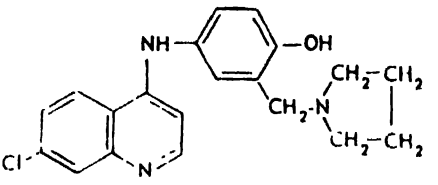


Amodiaquine
7-chloro-4-(3'-diethylaminomethyl-4'-hydroxyanilino)quinoline

There are well over 200 derivatives of 4-aminoquinoline with varying degrees of antimalarial activity. Among these sontoquine is less toxic than chloroquine, but also less active, and hydroxychloroquine has a lower chronic toxicity; but all of them, including cycloquine, have little advantage over chloroquine.

A variation on the chloroquine side chain was developed by French chemists, who produced a series of bis-quinolylpiperazines with the intention of extending the duration of the schizontocidal effect.

Amopyroquine, a pyrrolidine analogue of amodiaquine, is effective by mouth and parenterally against *P. falciparum* and *P. vivax* in man. It can be a substitute for amodiaquine in intramuscular administration.



Amopyroquine
7-chloro-4-(3'-pyrrolidyl-4'-hydroxyanilino)quinoline

One compound of the group of 4-aminobenzo[*g*]quinolines developed in the USSR has undergone clinical trials with promising results. The compound is known under the generic name of *dabechin*.

The emergence of strains of *P. falciparum* resistant to chloroquine has stimulated interest in new analogues of the 4-aminoquinolines, and there is some evidence of new 4-aminoquinolines which are effective against chloroquine-resistant malaria parasites.

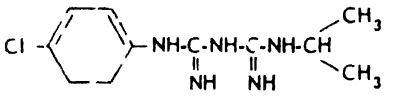
Biguanides

Research on new antimalarials carried out in Britain during the Second World War by Curd, Davey & Rose (1945) was based on the observation by Diaz de León that sulfonamides have some antimalarial action. Since sulfonamide derivatives of certain pyrimidines attain a high blood level, it was felt that these compounds might be introduced into other chemical structures. It was also thought that the activity of mepacrine was due to competition with riboflavine for some plasmodial enzyme systems. The characteristic of resonance (transition from one electron configuration into another of the same spatial structure of a molecule) was evident in the pyrimidines and in mepacrine. Pyrimidine derivatives carrying the dialkylaminoalkyl amino groups (characteristic of mepacrine and pamaquine) were prepared and gave rise to a long and ingenious series of modifications, each of the subsequent compounds being monitored for antiplasmodial action. It appeared eventually that, while a pyrimidine ring provided a convenient means for assessing all the possible relationships between structure and activity, a ring system was not essential; the biguanide molecule provided the necessary structural features around which active drugs might be prepared. Variations in the aryl substituents and in the terminal alkyl groups were then introduced. Compounds 4430 and 4888, in which a benzene ring was linked to a simple

isopropylamino group ($-NH-CH<^{CH_3}_{CH_3}$) through two amidine groups proved to be active

The highest antiplasmodial action was found in compound 4888 or *proguanil*, in which a biguanid chain has a chlorophenyl ring and a simple alkyl group (isopropyl) attached at each end

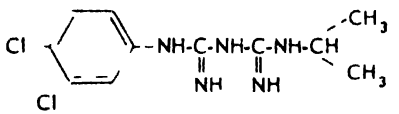
Proguanil
1-(*p*-chlorophenyl) 5 isopropylbiguanide



This compound, 1-(parachlorophenyl)-5-isopropylbiguanide, known as proguanil or chloroguanide (in the USA), proved to be more active than quinine in avian malaria and had little toxicity in laboratory animals It appears to attack the parasite by interfering with the nuclear division of its erythrocytic cycle through an inhibitory action on dihydrofolate reductase

Proguanil is one of many plasmodicidal drugs of this series The 3,4-dichloro analogue in the benzene ring yields chlorproguanil, which is more active than proguanil The bromine analogue of proguanil also has high activity, but chlorproguanil in particular has a more persistent action because of its less rapid excretion A number of other compounds (including nitroguanil, a relative of guanylurea) were prepared and showed a degree of activity but no advantage over proguanil

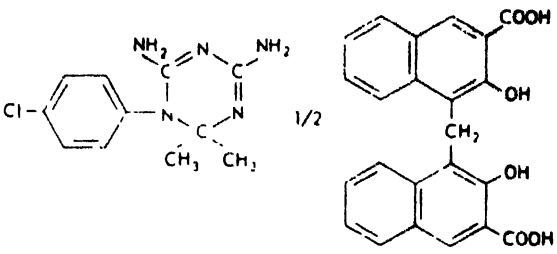
Chlorproguanil
1-(3,4-dichlorophenyl) 5-isopropylbiguanide



Proguanil exerts its antiplasmodial action indirectly through a metabolite produced by the host tissues (2,4-diamino-l-parachlorophenyl-1,6-dihydro-6,6-dimethyl-1,3,5 triazine) In man 60 % of the parent compound is excreted in the urine, 30 % of the drug as triazine Chlorproguanil is also metabolized to a triazine.

The biologically active metabolite of proguanil is *cycloguanil*, which bears a close structural relationship to pyrimethamine. The value of cycloguanil, the dihydrotriazine metabolite of proguanil, was explored by Thompson et al. (1965), who developed a long-acting injectable preparation consisting of the pamoic acid salt (embonate) of the parent compound. The prolonged action of this repository compound is attributable to the diffusion of the active portion of it (cycloguanil) from a depot at the site of injection. It was shown that up to 50 % of the injected drug remains for 2 weeks and small amounts

Cycloguanil embonate
4,6-diamino-1-(*p*-chlorophenyl)-1,2-dihydro-2, 2-dimethyl-*s*-triazine with 4,4'-methylene-bis(3-hydroxy-2-naphthoic acid) (2.1)

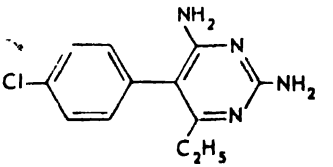


can be found for months. However, absorption of the drug depends on the particle size of the preparation and on the degree of local reaction to it.

Diaminopyrimidines

In the early 1940s, at the beginning of the intensive chemotherapeutic studies in the USA and the United Kingdom, the initial guidelines for synthetic variations were derived from sulfamethazine, a pyrimidine derivative with some antimalarial action. Some hybrids of this and similar structures with the dialkylaminoalkylamino side chain had a pronounced action in tests using avian plasmodia. Hitchings (1952) and his colleagues in the USA discovered an additional potent group of pyrimidines which proved to have a pronounced antagonistic effect on folic acid (pteroylglutamic acid) in cultures of *Lactobacillus casei*. The close structural relationship between 2,4-diamino-5-parachlorophenoxypyrimidine and the biguanides in their biologically active cyclic form led to the proliferation of a variety of allied compounds; those with a 5-phenyl substituent were the most active. Substitution of an alkyl group in the 6 position of the pyrimidine also increased the activity and, in the case of 5-parachlorophenyl derivatives, a high peak of antiplasmodial activity was obtained in animal trials with the 6-ethyl compound, which was given the generic name of *pyrimethamine*. Pyrimethamine and the active metabolite of proguanil have a structural analogy. Their antiplasmodial effect is due to the inhibition of dihydrofolic reductase, an enzyme that is essential for folate synthesis by the parasite, a process involved in the early formation of nucleic acids. Weight for weight, pyrimethamine proved to be many times more active than proguanil.

Pyrimethamine
2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine

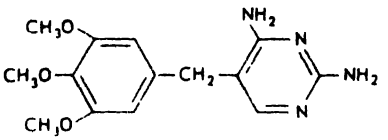


The basis for the selective action of pyrimethamine is differential binding to dihydrofolic reductase in different species of plasmodia and in the mammalian hosts and the extraordinary sensitivity to this action of the nuclear division of the malaria parasite at the time of the development of schizonts in

the erythrocytes and in the liver tissues. Pyrimethamine and proguanil are plasmodistatic rather than plasmodicidal drugs, leaving to the natural defence mechanisms of the body the elimination of the parasite whose growth is arrested.

The development of pyrimethamine was a brilliant feat of organic synthesis guided by biochemical considerations and (as some authors stressed) closer to Ehrlich's magic bullet than any of the other antimalarials. Several insoluble salts of pyrimethamine have been prepared with the aim of obtaining extended action. Additional chemical modifications of the 2,4-diamino-5-benzylpyrimidine structure led to the production of a 3,4,5-trimethoxybenzyl derivative known as *trimethoprim*, which shows a high degree of inhibition of bacterial dihydrofolate reductase and only a slight decrease in binding to the mammalian enzyme.

Trimethoprim
2,4-diamino-5-(3',4',5-trimethoxybenzyl)pyrimidine



Trimethoprim alone has some activity against certain species and strains of human plasmodia, but it is less effective than pyrimethamine. Host factors appear to play a part in the action of various combinations of this compound with other antimalarials.

Sulfones and sulfonamides

The discovery of the antibacterial action of sulfanilamide in the 1930s and the spectacular successes of the sulfonamides led to the synthesis and trial of a very large number of related compounds. The structural formula of sulfanilamide is:

Two types of compounds can be derived by substitution of the amide group (SO_2NH_2) or the amino group (NH_2). The most active sulfonamides belong to the first type, the amide group being replaced by a group usually with a heterocyclic ring. Later compounds of this series include sulfadiazine, sulfadimidine and sulfamethizole. Other compounds of this type were then developed that are strongly bound to plasma proteins and very slowly excreted; they are sulfamethoxypyridazine, sulfalene, sulfadimethoxine and sulfadoxine.

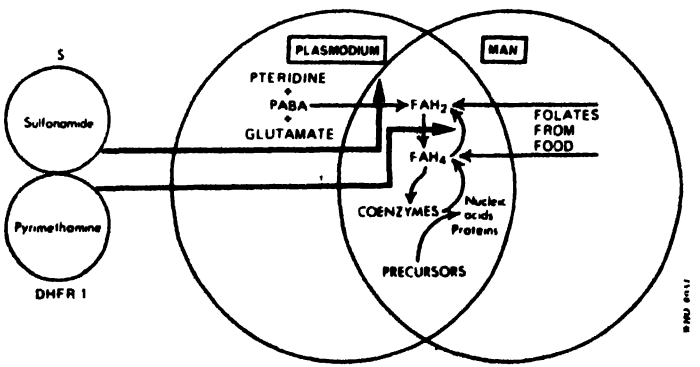
Compounds of the second type in which the amino group is masked must be activated in the body by removing the substituent group, since the free amino group is essential for antibacterial action. These compounds are split off in the intestine and the active substance is slowly liberated.

The antiplasmodial action of sulfonamides was reported as early as 1937 and many derivatives of these compounds were used with varying success against human malaria and experimentally in avian, rodent and simian infection. The slow rate and short duration of action and the need for high and potentially toxic doses were responsible for the shelving of these drugs, other more reliable compounds being available. However, the advent of the resistance of *P. falciparum* to 4-aminoquinolines in the 1960s has revived interest in the sulfones and sulfonamides.

Reports that malaria in patients with leprosy treated by *dapsone* (diaminodiphenylsulfone, DDS) was suppressed were in agreement with the finding that this compound shows very high activity in experimental rodent malaria. Early clinical studies confirmed the effect of dapsone on falciparum infections, although the action was slow. Following some success in using dapsone as an additional drug for the prevention of malaria in military contingents in southeast Asia, several derivatives of this compound were produced, aiming at a decrease in the rate of its metabolism and excretion from the body.

Wider acceptance of the combination of sulfonamides with antifolates was attributable not only to their successful use for various bacterial infections but also to understanding of the action involved. Already in the 1940s it had been postulated that sulfonamides compete with the structurally similar para-aminobenzoic acid for the same bacterial enzyme. Para-aminobenzoic acid is incorporated into the co-enzyme folic acid; inhibition of folic acid synthesis

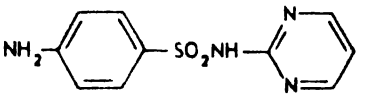
Fig 5 Diagrammatic representation of the potentiating action of sulfonamides and inhibitors of dihydrofolate reductase on malaria parasites[1]



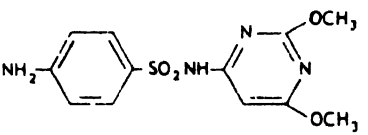
[1] Redrawn and amended from: Hitchings, G. H. (1978) In: Wood, C., ed., *Tropical medicine: from romance to reality*, London, Academic Press; New York, Grune and Stratton

by sulfonamides has been demonstrated and the enzyme involved was found to be tetrahydropteric acid synthetase. From the precursors (with which sulfonamides interfere) bacteria and plasmodia construct tetrahydrofolate, which all cells use as a co-factor in the formation of precursors of purines, needed for nucleic acid synthesis. In the process tetrahydrofolate is oxidized to dihydrofolate and must be regenerated by reduction. This process is brought about through the agency of the enzyme dihydrofolate reductase. Inhibition of this enzyme is a general property of the 2,4 diaminopyrimidines, which exhibit great selectivity since the amount needed to affect the metabolism of malaria parasites (or other micro-organisms) is several thousand times less than the amount that affects mammalian enzymes. The 2 active compounds combined act sequentially on the same metabolic pathway of the malaria parasite and this synergistic action is far greater than the additive effect of each of the 2 drugs (potentiation). Fig. 5 shows diagrammatically the difference between parasite and human folic acid metabolism and indicates the points at which sulfonamides and such folic acid antagonists as pyrimethamine or trimethoprim work.[3]

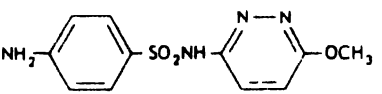
Sulfadiazine
N'-2-pyrimidinylsulfanilamide



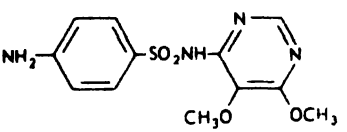
Sulfadimethoxine
N'-(2,6-dimethoxy-4-pyrimidinyl)-sulfanilamide



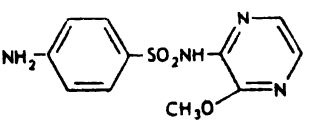
Sulfamethoxypyridazine
N'-(6-methoxy-3-pyridazinyl)-sulfanilamide



Sulfadoxine
N'-(5,6-dimethoxy-4-pyrimidinyl)-sulfanilamide



Sulfalene
N'-(3-methoxy-2-pyrazinyl)-sulfanilamide

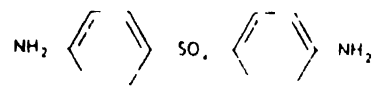


[3] The tentative suggestion that the combination of sulfones or sulfonamides with antifolate compounds (such as pyrimethamine or trimethoprim) should receive the generic name of sulfantifolate (or sulfantifol) has received a favourable initial response and may be more widely adopted.

The sulfonamides of main interest in malaria are the long-acting compounds with a half-life in the blood of between 60 and 200 hours such as *sulfadiazine*, *sulfadimethoxine*, *sulfamethoxypyridazine*, *sulfadoxine*, and *sulfalene*. The value of these drugs stems from the fact that they obviate the need for frequent administration.

The series of sulfones is represented by 4,4'-diaminodiphenylsulfone (dapsone, DDS) with a structural formula as follows:

Dapsone
4,4'-diaminodiphenylsulfone



Several derivatives of dapsone have been developed recently but there is no evidence that they are significantly better than the parent compound. Among them diformyldapsone (diformyldiaminodiphenylsulfone, DFD) and the injectable acedapsone (diacetylaminodiphenylsulfone, DADDS), with some repository activity, have shown some promise.

The absorption, distribution and excretion of sulfonamides and sulfones vary greatly with the structure of each compound and its solubility. When administered by mouth, most of the sulfonamides are fairly rapidly absorbed, peak concentrations in the blood being reached within 4 5 hours. An important factor in their subsequent distribution is the extent to which some are bound to plasma proteins (e.g. long-acting sulfonamides such as sulfadoxine or sulfalene). The drugs are cleared in 3 ways: partly by acetylation in the liver, partly by oxidation in the body and partly by excretion unchanged. The main route of excretion is by the kidneys. It takes well over 72 hours to eliminate a single dose of the long-acting compounds; this explains the serious adverse effects when these compounds are given at high doses or more frequently than necessary.

The results of the experimental treatment of human malaria transmitted to owl monkeys have shown that, when delivered in combination, a sulfonamide such as sulfadiazine and pyrimethamine enhance by 32 times the action of pyrimethamine and by 50–100 times the action of sulfadiazine when infection is by strains not resistant to either of the 2 drugs. In contrast to this result, the activity of the same drug combination on strains resistant to pyrimethamine was often disappointing. It is likely that this may occur in cases of falciparum malaria in man when the infecting strain is highly resistant to antifolate drugs. This is in accordance with the finding that sulfonamides and sulfones have a modest schizontocidal action against *P. falciparum* when used alone.

At the present time the combination of long-acting sulfonamides with antifolic compounds such as pyrimethamine is widely used for the treatment of falciparum malaria resistant to 4-aminoquinolines. Details of the administration of these drug combinations are given in Chapters 6 and 7.

Other compounds, including antibiotics

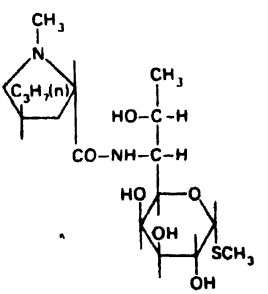
A large number of compounds have been developed and examined for antiplasmodial activity. They range from febrifugine, isolated from the powdered roots of *Dichroa febrifuga*, through its synthetic racemic 5-chloro derivative, several guanidines, pteridines, quinazolines, triazines, naphthoquinones, amidinureas, pyridines, pyrocatechols and organometallic substances (arsenicals, bismuth derivatives) to antibiotics and antimetabolites (actinomycin, cycloleucins, mitomycin, etc.). Few of these progressed beyond experimental testing on animal malaria and fewer still were extensively used on man. However, several very promising antimalarials have emerged from various investigations and especially from the large-scale scientific programme carried out during the past decade by the United States Army Research and Development Command These new developments in the chemotherapy of malaria are described in more detail in Chapter 4.

The present status of antibiotics for the treatment of malaria is briefly reviewed here.

Antibiotics. Since the discovery of penicillin in 1928 by Fleming and its isolation in a crystalline form by Chain & Florey (results published in 1941), several hundred antibiotics have been isolated, purified and synthesized. Among them the tetracyclines are a family of closely related antibiotics, the first of which was obtained from *Streptomyces aureofaciens*. Additions to the group of tetracyclines are still being made after 20 years.

The classification of antibiotics is complex and controversial. Their modes of action are very specific, three main mechanisms being in operation: inhibition of synthesis of the bacterial wall, increased permeability of cytoplasmic membranes, and interference with intracellular protein or nucleic acid synthesis. There is some correlation between the mode of action and the general spectrum of activity of antibiotics.

Already in 1952 Coatney & Greenberg had listed 31 antibiotics, of which tetracyclines, chloramphenicol, gliotoxin, fumigacin, tyrothricin and some others had some antiplasmodial activity. The first two of the above list had a slow therapeutic action on human malaria and a partial causal prophylactic effect on avian malaria. Prodigiosin and rifampicin have also been tried.



Lincomycin

Lincomycin, an antibiotic isolated from a soil streptomyces, is unlike any other major compound of this group; a number of synthetic derivatives including clindamycin have been prepared and are widely used for the treatment of various bacterial infections. According to the general consensus of experienced observers, only the tetracycline and lincomycin groups of antibiotics have any significant antiplasmodial action. Interest in compounds of the group of lincomycins was stimulated by demonstration of their activity against infections with chloroquine-resistant or dapsone-resistant strains of *P. berghei*. Further studies in sporozoite-induced and blood-induced infections with *P. cynomolgi* in the rhesus monkey showed that clindamycin had significant activity against early and late tissue schizonts, resulting in prevention or radical cure in a large proportion of infected animals. This compound and another derivative of chlorinated lincomycin were able to cure trophozoite-induced infections at well tolerated doses, and both compounds were equally effective against pyrimethamine-sensitive strains. Some of the new compounds were effective in infections with a highly resistant strain of *P. falciparum* in owl monkeys.

The tetracyclines have a wide range of activity against many bacterial species, mycoplasmas, rickettsiae and chlamydiae. However, an increasing number of microorganisms are showing an acquired resistance to tetracyclines.

Tetracyclines, usually in conjunction with quinine, have been reported to be suppressive in human malaria and proved to be of value as an additional drug for the radical cure of chloroquine-resistant falciparum infections. Another antibiotic, *clindamycin*, alone or in combination with quinine, has been used for the treatment of chloroquine-resistant falciparum malaria in Thailand with moderately good results, though not without adverse effects on the gastrointestinal tract. It is likely that it is one of the most potent antiplasmodial compounds among the antibiotics, but because of its toxicity it has a limited role as an antimalarial.

General Pharmacological Considerations

As a general rule, the chemotherapeutic action of a drug depends on the presence of an adequate concentration in the fluids circulating through the tissues and on the susceptibility of the target organisms to the drug. The concentration attained by the drug, in contact with the organisms on which it acts, depends on its absorption, distribution and clearance.

A drug may have to pass through a succession of cell membranes to reach its site of action in the body. The gastrointestinal tract, the tubules of the kidney, the sinusoids of the liver and the brain are surrounded by layers of cells controlling the uptake of substances into these organs; the cell membranes are essentially a double layer of oriented lipid molecules between two polypeptide layers. Drugs and nutrients pass across these membranes by various transfer

mechanisms such as diffusion through the lipid phase, filtration through pores, and active transfer by ionized carriers.

Absorption from the gastrointestinal tract depends on the function of this tract, which can be influenced by such factors as fever. The rate of absorption from an injection varies in relation to the type of the drug, its solubility and the physical characteristics of the preparation. Distribution of the drug within the body is related not only to the type of the compound but also to specific functions of the internal organs. Many drugs are bound to plasma proteins, particularly to the albumin fraction; this binding is reversible and there is a dynamic equilibrium between the bound and unbound form of the compound. The bound drug can be regarded as a storage depot since only the free form is active. This is of great practical importance in the use of some sulfonamides.

It is obvious that, for the effective treatment of an acute attack of malaria, a plasma concentration of the drug sufficient to affect the parasite in the red blood cells should be aimed at. The concentration of the drug in these cells may be much higher than that in the plasma.

Clearance of drugs from the body is effected in 2 ways: some are excreted unaltered but most are first metabolized and then excreted; some, however, are unaltered and fixed by specific tissues. While the liver is the most important organ concerned with the metabolism of drugs, the main organ of excretion is the kidney. The biochemical changes that drugs undergo in the body may lead to pharmacological activation or inactivation. Inactivation can occur through the processes of oxidation, reduction and hydrolysis and the rate of inactivation has an important bearing on the duration of the effect. Drugs can be inactivated by conjugation reactions, which are metabolic processes involving adenosine triphosphate, glucuronic acid, acetylation, etc. Drugs can also be transformed into active compounds; thus proguanil is oxidized to an active antiplasmodial metabolite. Many drugs are metabolized by enzymes located in the intracellular microsomes of liver cells. The rate of clearance of drugs varies enormously, the range extending from less than an hour to well over a week. Most drugs are cleared in an exponential manner, so that when a single dose of a drug is given the amount removed in unit time is a constant fraction of the amount still present. This implies that in practice it is impossible to produce prolonged action by giving a massive dose of a drug that is rapidly excreted. Prolonged action of such compounds can be obtained by delayed absorption or by frequent dosage (e.g. the quinine treatment of acute malaria when the drug is given every 6–8 hours). Cumulation results when the intake of a drug exceeds its clearance from the body. If a drug that is cleared in an exponential manner is given at regular intervals and if a constant fraction of the drug present in the body is cleared between intervals, it is easy to calculate the extent to which the drug will cumulate. Certain therapeutic agents have the exceptional power of slowly cumulating in some tissues; this is so for chloroquine, the concentration of which in the eye produces retinopathy and may have secondary effects in other tissues, e.g., skin (see p. 70).

The wide variations that occur in the fate of drugs in the human body explain the great variations in the frequency of drug administration. With most of the drugs that have a considerable therapeutic action it is necessary to produce an effective concentration in the blood as quickly as possible and to maintain that concentration for an adequate time. This is achieved on the principle of an initial "loading dose" followed by lower maintenance doses. Intravenous injection is often the only available method for rapid and intensive action; when a steady and more prolonged action is needed the intramuscular route is a suitable method, providing that the drug is tolerated at the site of injection. However, when a uniform concentration of the drug in the body fluids is sought, oral administration is still preferable, even though it is influenced by the bioavailability of the effective compound in relation to its pharmaceutical formulation. Thus, for instance, sugar-coated tablets of quinine, prepared with the intention of masking the bitter taste of the drug, may harden after some storage and will be absorbed only partly in the digestive tract. The same may occur with some paediatric formulations of other antimalarial compounds.

Knowledge of the rates at which drugs are metabolized and of the factors that influence this process are of considerable clinical importance. The half-life of a drug is defined as the time taken for its concentration in the plasma to fall by 50 %. This determines the duration of the drug's action and hence the optimum dosage interval. The response of many drugs is closely related to their plasma concentration, and the marked individual variations in response seen with standard doses can be greatly reduced if the dosage is adjusted on an individual basis.

Many factors influence drug metabolism. They include genetic constitution, age, nutritional state, previous or concurrent treatment with other drugs, and pathological conditions such as alcoholism, thyroid disease, liver disease and cardiac failure.

Expression of Dosages and Paediatric Prescribing

Doses of antimalarial drugs are commonly stated in fractions of a gram (g) or in milligrams (mg), as also the strength of tablets or other dosage forms such as injectable formulations. All antimalarial drugs in general use are organic bases and form salts with various acids. With the exception of some compounds (pyrimethamine, amodiaquine base), antimalarials are employed in the form of salts, since the acid component confers upon the drug several useful properties (ability to crystallize, stability and solubility) that are normally absent in a free base. Since only the base component is therapeutically active and since there are differences in the proportion of base contained in various salts, doses of antimalarial drugs should preferably be expressed in terms of base. Most manufacturers indicate either the content of base in their antimalarial drugs or give both the salt and the base content. However,

quinine is commonly prescribed in terms of the salt, even though 1 gram of the hydrochloride or dihydrochloride contains 82 % of active base while the same amount of bisulfate contains only 59 %.

The dosage of older drugs has been generally accepted and has been adopted as a result of clinical experience; newer drugs have undergone systematic study in clinical and therapeutic trials. Guidance given by the national pharmacopoeias varies; thus the British Pharmacopoeia gives the range of doses for drugs, whereas the United States Pharmacopeia gives the usual dose in addition to the range. These doses are calculated for adults on the assumption of a body weight of 65–70 kg. However, there is individual variation in the response to drugs and this must always be kept in mind, especially in extremes of the physical constitution of the patient and in severe illness. The aged are less able to metabolize drugs and for them a marginally lower dose should be used. Wherever the dosage of drugs has to be adjusted to the individual patient, as for the aged or children, the best correlation is with the surface area of the body. However, for infants there is no fully reliable formula, since metabolism and excretion are not fully developed in infants. The dosage for children is often calculated by various formulae, of which that of Young is the best known:

$$\text{child's dose} = \text{adult dose} \times \frac{\text{age of child in years}}{\text{age of child in years} + 12}$$

None of the formulae for calculating the paediatric dosage is completely satisfactory, for all assume that at least some measurements—the child's age, weight or body surface area—can be accurately gauged. The age is often a matter of dispute and the weight may be distorted by oedema or wasting, while the calculation of the dosage from the body surface area is too complicated for ordinary purposes. The best compromise is to adopt the percentage method advocated by experienced paediatricians (see Table 2).

TABLE 2 WEIGHT PERCENTAGE METHOD FOR PAEDIATRIC PRESCRIBING

Weight of child[1] kg	Percentage of adult dose (assuming the weight of adult to be 65 kg)
4 5	15 0
10 0	25 0
15 0	33 3
23 0	50 0
30 0–40 0	75 0
45 0–65 0	100 0

[1] These are approximate median weights, corresponding roughly to the following age groups 1–6 months, 6–12 months, 2–4 years, 4–7 years 7–11 years, and 11–16 years

Another rough and ready method for reckoning the dosage for children is based on proportions according to age. It is as follows:

Age of child	*Proportion of adult dose*
Infants up to 2 years	1/8 to 1/4
Children 2–6 years	1/4 to 1/2
Children 6–12 years	1/2 to 3/4
Over 12 years	3/4 to 1

Absolute precision in paediatric prescribing is impossible in general practice. An oedematous or very fat child needs a dosage at the higher limit of the range, an undernourished one at the lower limit. If there is no evidence of liver or kidney disease it is safe to round the dose up. It is always, however, advisable to check the calculations against the manufacturer's leaflet. In very sick children absorption from the gastrointestinal tract may be greatly delayed and a tendency to vomiting may vitiate the intended dosage. Parenteral administration of antimalarials solves this problem, provided that the correct dosage is given. However, both intravenous and intramuscular injections of certain antimalarials in children may present some danger; it may be prudent to give half of the intended dose, then the other half 1–2 hours later. Intravenous injections must be given very slowly, and an intravenous infusion in glucose–saline is nearly always preferable.

Nomenclature of Antimalarial Drugs and Monitoring of Adverse Effects

Most antimalarial drugs are known under a variety of names, and this is responsible for some confusion. New drugs, if considered suitable for general use, are put on the market under a manufacturer's proprietary (or brand) name, which is registered as a trade mark. Whenever the new drug is a commercial success, alternative methods of production are usually developed by competitors; these methods evade the restrictions imposed by patents, and sooner or later the new compound is advertised under several different proprietary names. Moreover, a series of new compounds may be produced, with similar medicinal action but to some extent different chemical constitution. These are advertised under different proprietary names and add to the confusing nomenclature of new drugs.

After a new drug has been shown to have a likely application it is given an approved name. Approved names are nonproprietary names for new drugs devised or selected by a national pharmacopoeia commission or similar body. The approved name is usually based on a contraction of the full chemical name and is often difficult to remember and to write. Thus for convenience as well as for commercial reasons the drug is released under its proprietary name. This makes it difficult to establish an approved name in general use, even though a drug under an approved name is often less expensive.

Under certain conditions, which amount to an official endorsement of their value (not necessarily a greater value than that of other compounds), new drugs are admitted to a national pharmacopoeia and to the International Pharmacopoeia. They are then given a nonproprietary name, which is formally accepted. Naturally, all compounds are defined by their own chemical names, which indicate their fundamental chemical structure. Series of drugs of similar molecular structure and action are often given under the name of their generic chemical group (e.g. 4-aminoquinolines).

In addition to various proprietary or trade names, a drug may be known in different countries under different nonproprietary names. For example, the drug commonly known as proguanil was originally known under the code number M4888; it was then released on the market under the trade name Paludrine, which is the property of the company concerned; and it is entered in the British Pharmacopoeia under the name proguanil, which anyone can use. This name was later accepted as the International Nonproprietary Name (INN) and it is now the correct official name of this substance. However, in the USA the nonproprietary name of proguanil is chlorguanide and in the USSR bigumal, and there are at least a dozen different trade names for this compound.

When trade names are used they are normally written with a capital initial letter (e.g. Paludrine). In the USA the protected status of such names is indicated by the addition of a sign ® indicating a trademark. Nonproprietary names are spelled with a small letter (e.g. proguanil).

The selection of International Nonproprietary Names (INN) for pharmaceutical substances has been coordinated by WHO since 1949. Proposals for recommended INN are periodically submitted to WHO and adopted or not according to well established principles.

International Nonproprietary Names should be used in preference to any other names for antimalarial drugs except when it is desired to refer specifically to the product of a particular manufacturer. According to the general principles for guidance in devising such names the following suffixes are used: "ine" for alkaloids and organic bases; "quine" (Russian "hin") for drugs containing a quinoline group; and "crine" for drugs containing an acridine group.[4]

Drug monitoring

The introduction of new drugs faces today a number of problems, owing to increasing intervention by national governments with the avowed purpose of protecting the interests of the public by reducing the high cost and excessive multiplication of some compounds and the adverse reactions that some cause.

[4] WHO Technical Report Series, No. 581, 1975 (*Nonproprietary names for pharmaceutical substances*. Twentieth report of the WHO Expert Committee).

A number of advanced countries have now adopted various methods of monitoring the possible adverse effects of new drugs, and all major pharmaceutical companies now carry out what is called "post-marketing surveillance". An international drug monitoring system was proposed by the WHO in 1971. The main objective of such monitoring systems is to decrease the time lag between the introduction of a new drug into general medical use and the discovery that it produces an adverse reaction that was not expected on the basis of the preliminary clinical trials, which are of necessity limited to a relatively small number of people.

The adverse effects of antimalarial drugs are relatively few, though some, such as the haemolytic effects of primaquine or sulfones in persons with G6PD deficiency, may be more frequent in some parts of the world. Problems related to the long-term administration of prophylactic compounds have recently been given more attention. A detailed review of the adverse effects of antimalarial compounds will be found in Chapter 6.

During the past decade there has been a marked increase in the number of pharmaceutical products marketed. This has resulted in a plethora of similar, if not identical, drugs appearing under a bewildering variety of trade names, and often there is doubt about the choice of the appropriate one. It is clear that, to make the best use of the limited financial resources of developing countries, where communicable diseases are particularly common, the number of drugs in those countries with different names but with the same properties should be restricted. WHO has recently proposed that a number of essential generic drugs of primary importance should be selected, but the acceptance of such a list depends entirely on the decision of the countries concerned.

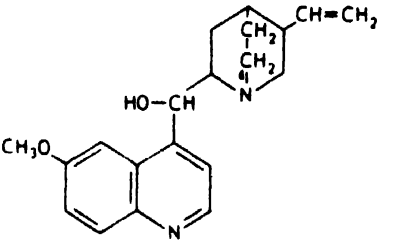
CHAPTER 3

PHARMACOLOGY OF COMPOUNDS IN CURRENT USE

Individual Compounds[1]

Quinine

Structure



Quinine
6-methoxy α-
(5-vinyl-2-quinuclidinyl)-
4-quinoline methanol

Spectrum of activity in human malaria

(1) Sporozoites
(2) Primary exoerythrocytic stages
} Inactive Quinine is not suitable for *causal prophylaxis*

(3) Asexual erythrocytic stages Quinine is a highly active blood schizontocide in all forms of human malaria, and hence an effective drug for clinical cure With prolonged administration *radical cure* is often possible in falciparum infection, but seldom in vivax infection For *suppression* and *suppressive cure* quinine is less efficient than the 4-aminoquinolines or mepacrine

(4) Gametocytes Quinine has some activity against immature but not against mature gametocytes in falciparum malaria It is an effective gametocytocide in vivax, ovale, and quartan malaria For general *gametocytocidal prophylaxis* it is a poor drug

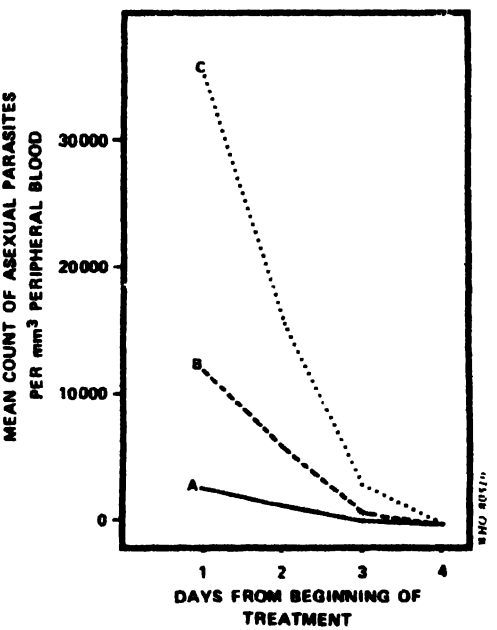
[1]The dosage, routes of administration and adverse effects of these compounds are dealt with in Chapter 6 Annex 2 contains a list of synonyms, Annex 3 further data on formulations, and Annex 4 information on tests for the presence of antimalarials in biological fluids

(5) Latent exoerythrocytic stages: Inactive. Quinine thus cannot effect a *radical cure* in vivax malaria when used alone; however, when combined with 8-aminoquinolines a high cure rate is possible in vivax infection.

(6) General: The value of quinine in the treatment of malaria is attested by long experience. However, because of its relative toxicity in therapeutic dosage it has been replaced by the 4-aminoquinolines for the *radical cure* of quartan malaria and of falciparum malaria in areas where the parasite remains sensitive to chloroquine. Quinine has also been replaced by the 4-aminoquinolines for the treatment of acute vivax or ovale malaria, although none of these drugs will produce a radical cure as they lack activity against the latent tissue stages (hypnozoites).

Consistent in action on the schizogonic blood forms, quinine may be used with confidence for the relief of symptoms in acute malaria (Fig. 6 and 7).

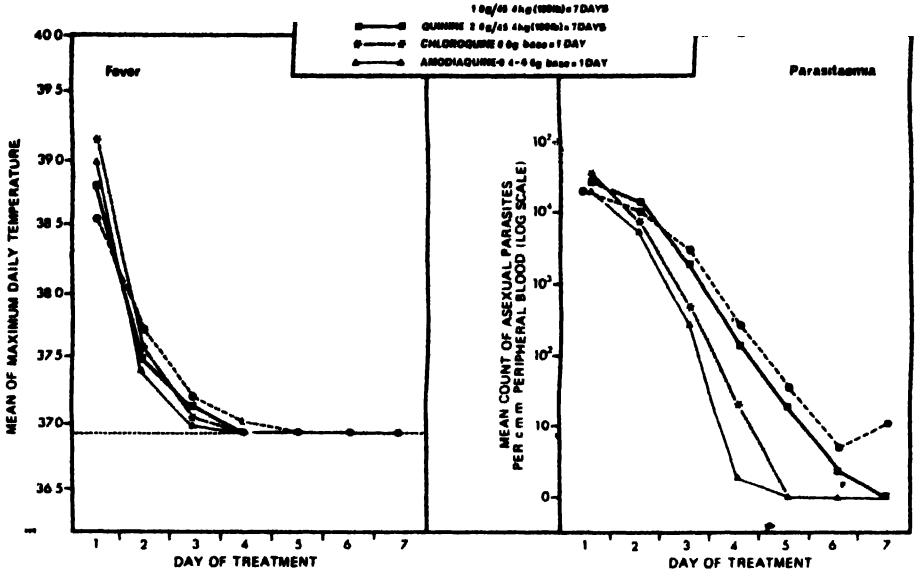
Fig. 6. Effect on asexual parasitaemia in acute malaria of quinine dihydrochloride in doses of 1·0 to 2·0 g per 45 kg body weight



A = *P. malariae* 32, B = *P. vivax* 293, C = *P. falciparum* 645 cases
Information from the Malaria Research Division, Institute for Medical Research, Malaysia, 1946–47.

Some authorities still adhere to the view that quinine is unsurpassed by any of the new synthetic compounds for the immediate treatment of severe falciparum infection. This view is particularly acceptable in those areas where

Fig. 7. The effect of quinine, chloroquine and amodiaquine on fever and parasitaemia in acute falciparum malaria in patients whose pretreatment parasite counts were below 100 000 per mm³ [1]



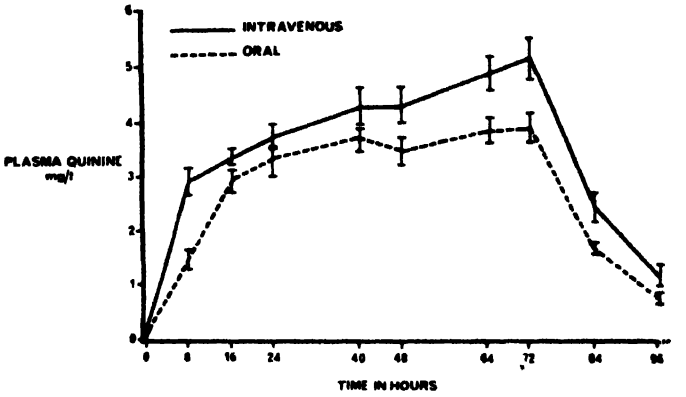
[1] From: Wilson, T & Edeson, J F. B (1958) *Medical journal of Malaya*, 12 472

the sensitivity of falciparum malaria to the 4-aminoquinolines has decreased. Because most chloroquine-resistant strains of *P. falciparum* retain their sensitivity to quinine, quinine is considered at present a drug of choice for the treatment of the acute attack of malaria in such localities (Chapter 6).

Pharmacokinetics

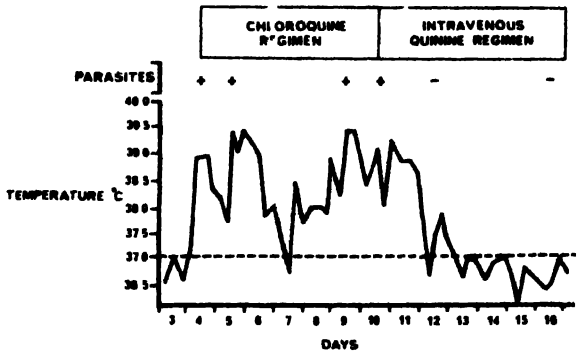
Quinine passes through the stomach unchanged and is quickly and almost completely absorbed from the upper intestinal tract to circulate as a base. While the plasma level is affected by the route of administration, the drug appears in the urine within an hour or less whether given by the mouth or by intramuscular or intravenous injection. Quinine is quickly metabolized by the tissues or excreted unchanged in the urine, and little remains in the body 48 hours after the last dose, its half-life being about 10 hours. Peak concentrations in the plasma are reached 1–3 hours after a single oral dose, the amount in the red cells being about one-fifth of that in the plasma. A mean plasma concentration of 2–5 mg/l is probably necessary to reduce parasitaemia in acute vivax malaria, and 5 mg/l to eliminate asexual parasites from the blood stream (Fig. 8); concentrations lower than 2 mg/l have little effect Somewhat higher concentrations are usually necessary in falciparum

Fig 8 Comparison of mean plasma quinine levels in 22 healthy volunteers receiving oral quinine dihydrochloride (1 62 g base daily for 3 days in 3 divided doses), and 11 receiving intravenous infusion for 72 hours (0 49 g base in 500 ml 9 g l NaCl each 8 hours totalling 1 47 g in each 24 hours)[1]



Significantly higher levels were reached by the intravenous route at 48, 64, and 72 hours (Vertical bars = means ± S E)
From Hall A P et al (1973) *Clinical pharmacology and therapeutics*, **27** 66

Fig 9 Influence of quinine dihydrochloride in continuous infusion (1 8 g in 1 5 l normal saline over a 24-hour period, repeated for 10 days, total 14 7 g quinine base) in an adult male during the third recrudescence of *P falciparum* following chloroquine therapy[1]



[1] The parasitaemia was not affected by chloroquine during the fourth attack but intravenous quinine resulted in a radical cure
From Hall, A P, Arnold, J D & Martin, D C (1974) *Southeast Asian journal of tropical medicine and public health*, **5** 128

infection. With both species the effective plasma level may depend on the strain of parasite (Fig. 9).

Tolerance and toxicity

Quinine has its own characteristic side effects. Giddiness, ringing in the ears, tremors and blurred vision may occur during the first few days of administration in some persons, but these symptoms usually subside when administration of the drug ceases. Idiosyncrasy to quinine with more serious symptoms occurs but is rare.

Solutions of quinine are caustic and must not be injected into the subcutaneous tissue if the vein is accidentally missed. Intramuscular injections may leave fibrotic indurations *in situ* that last for a considerable time. Faulty technique in intramuscular injection may lead to other more serious complications if complete sterility of the apparatus used is not assured. However, with sensible precautions the risks of intramuscular injections are minimal.

Given intravenously, quinine lowers the blood pressure. Fatal collapse may occur as a result of intravenous injections administered too quickly in very severe infection. Quinine may be given safely by intravenous infusion in a physiological saline drip. Whether the drug is given orally or parenterally, ideally the plasma level should be monitored in patients who have defective renal function in order to avoid the development of an unduly high blood concentration of quinine.

The very bitter taste of quinine may cause difficulty with oral administration to children. Preparations more acceptable to children are available, but are usually expensive.

Contraindications

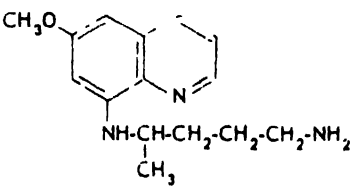
There are few contraindications to the use of quinine in the treatment of malaria. Patients with an idiosyncrasy to quinine must obviously be treated with some other drug. Haemoglobinuria and anuria may be caused by the drug in some individuals. A history or threat of blackwater fever could be an indication for withholding quinine in favour of one of the synthetic compounds. The question of quinine in pregnancy has lost its urgency with modern developments in therapy; untreated malaria is more likely than quinine to induce abortion, but most physicians make assurance doubly sure by using some alternative drug effective against the local strain of *P. falciparum*.

Salts in common use

Sulfate, bisulfate, hydrochloride and dihydrochloride, prescribed either in solution or in tablets or capsules. (Sugar-coated tablets may fail to dissolve and should be avoided). See Annex 3.

Primaquine and other 8-aminoquinolines

Structure:



Primaquine
6-methoxy-8 (4'-amino-
1'-methylbutylamino)quinoline

Spectrum of activity in human malaria

(1) Sporozoites: Probably inactive.

(2) Primary exoerythrocytic stages: Active against these stages of *P. vivax* and *P. falciparum*, particularly the latter. The activity against those of *P. malariae* is not known.

(3) Asexual blood stages: Active against the asexual blood forms of *P. vivax* and *P. falciparum*, but only at a dosage dangerously high for routine use.

(4) Gametocytes: *Highly gametocytocidal* against all species of human malaria parasite.

(5) Latent exoerythrocytic stages: Highly active. *Radical cure* of vivax malaria is usually produced whether the drug is administered during a relapse or during latency.

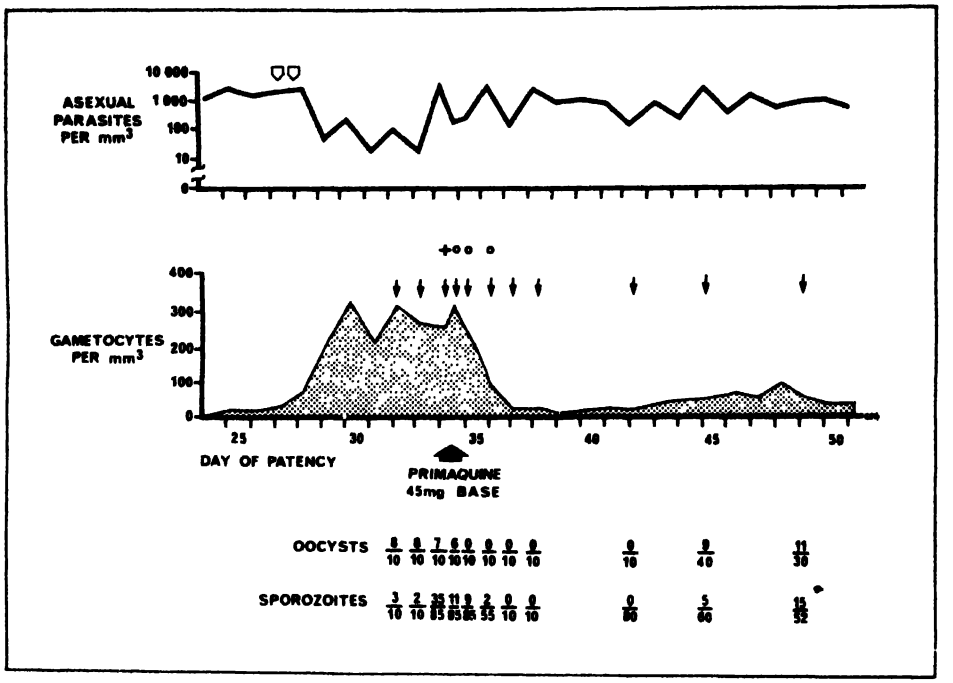
(6) General: The discovery in primaquine of a drug that could act as a causal prophylactic, destroy the gametocytes of all species of parasite as well as the asexual blood forms, effect radical cure of an established infection, and be produced without difficulty at relatively low cost raised the hope that the ideal remedy for malaria had been found. The fact that its action on asexual blood parasites, like that of the other 8-aminoquinolines, is effective only at dangerously high dosage has, however, precluded its use for treatment of the acute attack, though it has been used extensively for the prevention of relapses of vivax malaria, for which until now it is the main drug available.

Primaquine inhibits parasite mitochondrial respiration and this is probably the basis of its action against the primary and secondary liver stages as well as against gametocytes. The gametocytocidal action of a single 45-mg dose of primaquine base against *P. falciparum* lasts for several days, rendering the gametocytes incapable of development in mosquitos that subsequently feed on the treated patient (Fig. 10).

Pharmacokinetics

The 8-aminoquinolines are rapidly absorbed from the gastrointestinal tract and rapidly excreted. A single dose of primaquine is eliminated in the urine in

Fig. 10. Gametocytocidal and sporontocidal effects of primaquine on a chloroquine-resistant strain of *P. falciparum*[1]



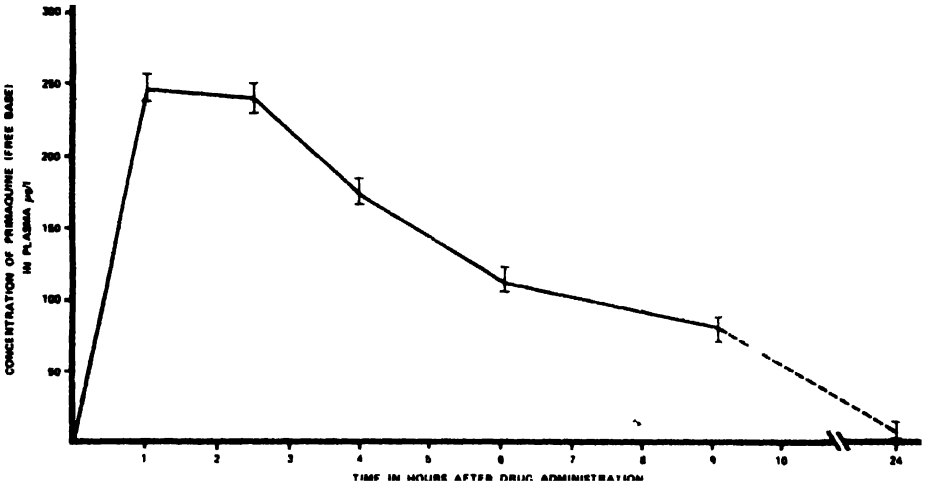
[1] The open arrows indicate administration of quinine in doses of 540 mg; the small solid arrows indicate times when batches of *Anopheles stephensi* were fed; + or 0 indicates success or failure of attempts to transmit infections with a particular group of mosquitos. Note the prolonged inhibitory action of the single dose of 45 mg primaquine base on sporogony in this nonimmune volunteer.
From: Rieckmann, K. H et al (1968) *Bulletin of the World Health Organization*, **38**: 625.

the form of metabolic degradation products within 24 hours, and only a very small amount is fixed in the tissues. Recent unpublished studies have shown that a mean blood level of 250 μg/l is reached within 1 hour of ingesting a single dose of 45 mg primaquine base (Fig. 11). The plasma concentration falls rapidly to about half this level within 6 hours. The structure of the metabolic products in man has not yet been determined. It has been postulated that pamaquine (an early, but now obsolete member of the 8-aminoquinoline series) is converted to an active 5,6-quinolinequinone metabolite.

Tolerance and toxicity

At the recommended dosage no symptoms of toxicity are likely. When the dosage is increased, toxic manifestations may include anorexia, nausea,

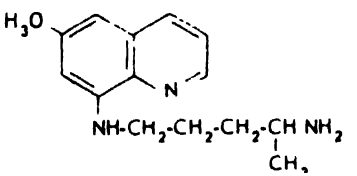
Fig. 11 Mean blood levels of primaquine in man after ingestion of a single dose of 45 mg base[1]



[1] Unpublished data from Dr K A Fletcher

cyanosis, epigastric distress, abdominal pain and cramps, the passage of dark urine and, occasionally, vomiting, vague chest pain, and weakness. In addition, there may be striking effects on the formed elements of the blood and the bone marrow, marked by leukopenia, anaemia, methaemoglobinaemia and suppression of myeloid activity, with lesser effects on the heart and circulation. These manifestations disappear when the drug is withdrawn.

Quinocide, a congener of primaquine, is stated to be somewhat more toxic than primaquine.

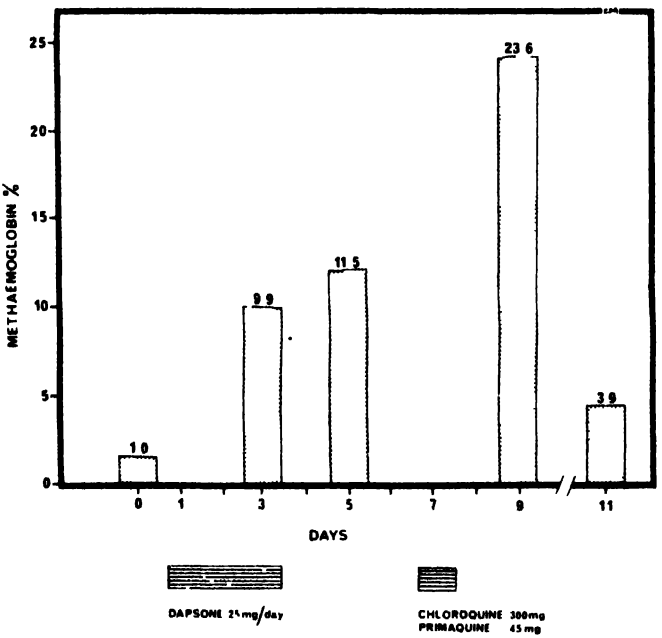


Quinocide
6-methoxy-8-(4'-amino-4'-methylbutylamino)quinoline

The haemolytic action of primaquine and other 8-aminoquinolines is related to the presence of certain hereditary enzyme deficiencies, particularly that of glucose-6-phosphate dehydrogenase (G6PD). Marked haemolysis occurs when primaquine is administered daily to individuals with G6PD deficiency, owing to destruction of the older erythrocytes. Consequently, a single dose of 45 mg weekly is better tolerated than daily doses of 15 mg base by G6PD-deficient subjects. Primaquine can produce methaemoglobinaemia (manifested by cyanosis) in normal individuals, but the effect is most marked

in those with congenital deficiency of nicotinamide adenine dinucleotide (NADH) methaemoglobin reductase (Fig. 12). Sulfonamides, dapsone and other oxidant drugs can also cause haemolysis or methaemoglobinaemia in enzyme-deficient subjects.

Fig 12 Methaemoglobinaemia provoked by dapsone followed by chloroquine and primaquine in a subject deficient in erythrocyte NADH methaemoglobin reductase[1]



[1] From Cohen, R J et al (1968) *New England journal of medicine*, **279** 1127

Contraindications

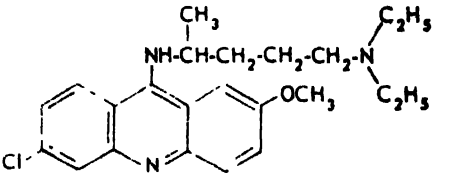
Primaquine should be administered with caution in subjects known to have the hereditary enzyme deficiencies mentioned above. It is advisable also not to administer it during the first trimester of pregnancy for the radical cure of vivax malaria, but to treat any relapse that may occur with chloroquine and give a course of primaquine later during pregnancy or after delivery. The 8-aminoquinolines are also contraindicated in patients with a tendency to granulocytopenia.

Salts in common use

Primaquine diphosphate; quinocide dihydrochloride (see Annex 3).

Mepacrine

Structure:



Mepacrine
2-methoxy-6-chloro-9-
(4'-diethylamino-1'-methylbutylamino)acridine

Activity in human malaria

(1) Stages of life cycle: See chloroquine and other 4-aminoquinolines.

(2) General. Mepacrine is largely obsolete as an antimalarial. Although it has distinct advantages over quinine, these are offset by the following limitations: (i) it dyes the skin and conjunctivae yellow and occasionally causes alarming, though transient, mental disturbances; (ii) it must be given daily, or not less than twice weekly, for complete suppression of all types of malaria; (iii) adequate management of acute malarial attacks in nonimmune subjects may require 7 days of drug administration; (iv) strains of *P. falciparum* that are resistant to the 4-aminoquinolines exhibit cross-resistance to mepacrine.

Stocks of mepacrine are still available in certain countries where it is used for indications other than malaria (e.g. the treatment of giardiasis and of various helminthiases). While mepacrine should no longer be used for the prevention or treatment of malaria if safer and more effective drugs such as chloroquine are available, it can be employed in an emergency if it happens to be the only compound on hand. For this reason the following information is provided.

Pharmacokinetics

Absorption of the drug is rapid. Elimination is slow, owing to its pronounced affinity for the organs and tissues, where the concentration after therapeutic doses may reach several hundred times that found in the plasma; only about 10 % or less is eliminated in the urine daily, with the result that 3–4 weeks are required for concentrations to drop below non-effective levels. Peak concentrations of 50–60 μg/l of plasma are attained during the first day of oral administration of loading doses. When doses of 200 mg are given

intramuscularly, therapeutically effective plasma mepacrine concentrations are attained within 15 minutes

Tolerance and toxicity

When administered initially at the recommended dosage, gastrointestinal distress may appear in the form of abdominal cramps, nausea, vomiting and diarrhoea, but these symptoms disappear with continued administration of the drug Various mental symptoms may occasionally occur, but disappear when the drug is withdrawn Especially under humid tropical conditions cutaneous lesions may develop into a generalized exfoliating dermatitis

Contraindications

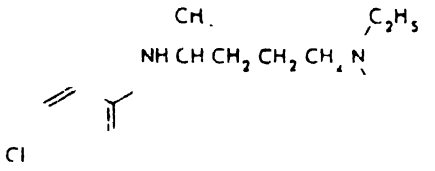
Apart from individual idiosyncrasy syphilis affecting the central nervous system is the only contraindication to the use of mepacrine in the treatment of malaria Convulsive seizures have been recorded in such patients following its administration and it is advisable, therefore, to use some other drug For radical treatment 8-aminoquinolines may be given after the completion of a standard mepacrine regimen, but simultaneous administration of mepacrine and 8-aminoquinolines is contraindicated

Salts in common use

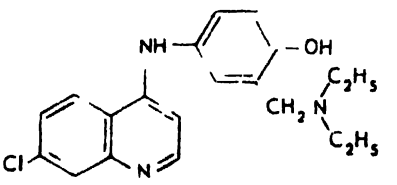
Dihydrochloride dihydrate, methane sulfonate for injection See Annex 3

Chloroquine and other 4-aminoquinolines

Structures

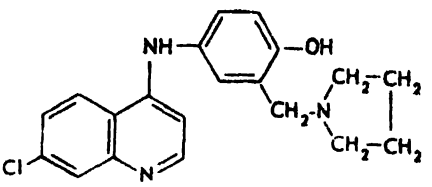


Chloroquine
7-chloro-4 (4 diethylamino
1 -methylbutylamino)quinoline

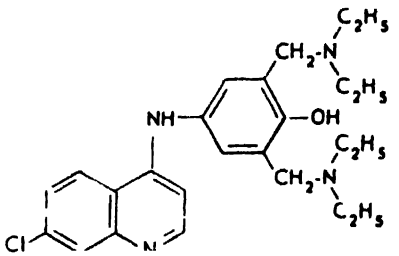


Amodiaquine
7-chloro-4 (3 -diethylaminomethyl
4 -hydroxyanilino)quinoline

Amopyroquine
7-chloro-4-(3'-pyrrolidyl-4'-hydroxyanilino)quinoline



Cycloquine
7-chloro-4-[3',5'-bis(diethylaminomethyl)-4'-hydroxyanilino]quinoline



Spectrum of activity in human malaria

(1) Sporozoites:
(2) Primary exoerythrocytic stages: } Inactive

(3) Asexual blood stages: Chloroquine and other 4-aminoquinolines are highly effective against the asexual blood stages of all four species of malaria parasite except in areas where drug-resistant strains occur. They produce *clinical cure* of all types of human malaria and *radical cure* of falciparum and quartan infections. They are excellent *suppressive* agents against all species; administration continued for 4–6 weeks results in *suppressive cure* of falciparum and quartan malaria. Morphological changes, marked by distinctive clumping of pigment, are characteristic of their action. These changes are observed in asexual blood forms at all stages of development.

(4) Gametocytes: Chloroquine and other 4-aminoquinolines act as *gametocytocidal agents* against *P. vivax*, *P. ovale* and *P. malariae* and are effective against the immature but ineffective against the mature gametocytes of *P. falciparum*.

(5) Latent exoerythrocytic stages: Inactive They do not produce *radical or suppressive cure* of infections caused by *P. vivax* or *P. ovale*.

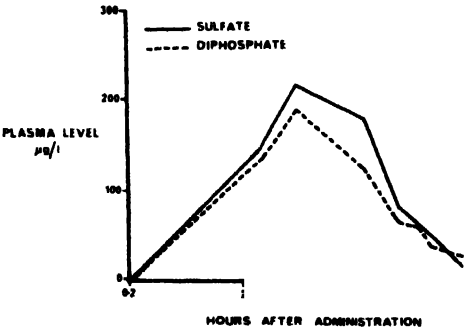
(6) General: Chloroquine, amodiaquine and cycloquine have replaced other blood schizontocides as the drugs of choice for the treatment of acute malaria. Their action is rapid, fever usually being absent after 24 hours and patent parasitaemia being eliminated in 48–72 hours following the standard therapeutic regimen (see Chapter 6). If the response in falciparum infection is slow or incomplete, drug resistance should be suspected (see Chapter 5). Amodiaquine and amopyroquine may have a slight advantage over chloroquine in such cases. Amopyroquine, unlike amodiaquine, can be administered parenterally.

Pharmacokinetics

Chloroquine, amodiaquine and cycloquine are rapidly and almost completely absorbed from the gastrointestinal tract. They are extensively localized in the tissues, where they become concentrated in lysosomes, particularly in such cells as those of the liver parenchyma. They are also selectively concentrated in melanin-containing organs. From all these sites they are slowly excreted and metabolized. When a loading dose is employed, an effective chloroquine concentration is reached within 2–3 hours, and after intramuscular administration within about 15 minutes. The therapeutically effective plasma concentration in man appears to be of the order of 30 μg base/l against drug-sensitive *P. falciparum* and 15 μg/l against *P. vivax*. The 4-aminoquinolines are also selectively concentrated in malaria-infected erythrocytes, the level of concentration in chloroquine-sensitive *P. falciparum* infections being significantly greater than in those with drug-resistant parasites. The minimum concentrations of 4-aminoquinolines required to inhibit the maturation of blood schizonts of a drug-sensitive strain of *P. falciparum* (e.g. strain Uganda 1) *in vitro* are chloroquine 250, amodiaquine 50 and amopyroquine 50 μg salt/l of blood. Corresponding figures for the chloroquine-resistant Viet Nam (Marks) strain are 2500, 100 and 100 μg salt/l (see Table 3, p. 75).

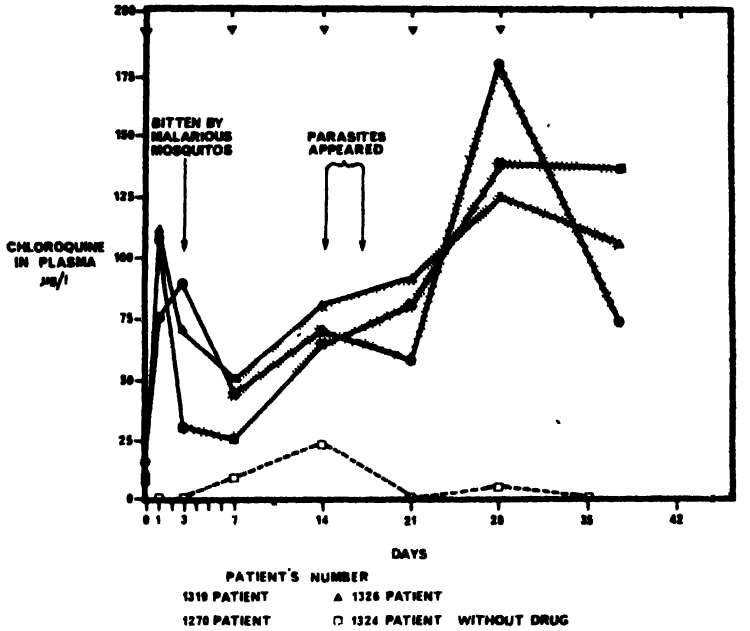
Most of the chloroquine in the plasma (Fig. 13 and 14) and tissues remains as the parent compound, but metabolites are formed by degradation of the alkylamino side chain. In daily administration, about 10% of the drug is excreted in the faeces and 56% in the urine. In the latter the unchanged parent

Fig 13 Plasma levels of chloroquine following a single dose in man 0 6 g base given orally as diphosphate or sulfate to 5 subjects in a cross-over design trial[1]



The diphosphate was given first and the sulfate 4 weeks later Plasma chloroquine levels assayed by the Brodie et al (1947) technique From McChesney, E W, Banks, W F & McAuliff, J P (1962) *Antibiotics and chemotherapy*, **12** 583

Fig 14 Plasma levels of chloroquine in 4 patients receiving 300 mg base weekly, infected with the Colombia strain of *P. falciparum* by mosquito bite[1]



[1] The shaded area indicates the range of plasma chloroquine levels that failed to suppress asexual parasitaemia in these individuals

From Young, M D (1962) *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **56** 252

compound represents 60–70% of the identifiable material, while 23–37% consists of secondary amines and about 3% of the primary amine, 4-amino-7-chloroquinoline. Some of the metabolites may also be active antimalarials.

Tolerance and toxicity

The toxicity is minimal at the doses ordinarily employed for therapy or suppression. Although headache, pruritus and blurring of vision have been reported following therapeutic doses, these symptoms usually disappear soon after administration has been discontinued.

Chloroquine given rapidly by the intravenous route can cause an abrupt fall in blood pressure, which may be fatal. The drug is rapidly absorbed when given intramuscularly; it is therefore rarely necessary to give it intravenously, but if intravenous administration is considered it should be administered slowly in a saline drip.

The side effects of the 4-aminoquinolines have usually followed the prolonged administration of large doses (e.g. 300–600 mg base daily) for weeks or months. Ocular damage may take the form of neuroretinitis, which is probably related to the high affinity of melanin-containing tissues for the 4-aminoquinolines. It has been suggested that a cumulative dose of 100 g chloroquine base over 2½–3½ years is the maximum quantity that can safely be taken. If diagnosed early some ocular lesions may be reversible.

Skin lesions may take several forms, which range from severe pruritus to various types of pigmentation. Some Africans may develop pruritus after taking the doses of chloroquine normally recommended for treatment; however, the symptoms rapidly disappear on withdrawal of the drug. Pigmentation of the nail bed and palate has been reported in some people taking amodiaquine over long periods.

It must be emphasized that serious side effects such as those related to the eye and the skin have only very rarely been reported in individuals receiving the 4-aminoquinolines for malaria suppression or therapy in the standard recommended doses.

Contraindications

Few contraindications have been reported to date except in some sensitive individuals who develop severe pruritus on being treated with chloroquine. The 4-aminoquinolines should be avoided by those with a history of collagen disease who have already taken a large cumulative dose of these or allied compounds and by any individual with a history of ocular or skin reactions to such drugs. There have been no reports of teratogenicity associated with chloroquine as an antimalarial, and it can therefore safely be recommended to women of child-bearing age or during pregnancy

Alternative drugs should be considered in areas where strains of *P. falciparum* resistant to the 4-aminoquinolines are known to occur.

Because of the bitter taste of 4-aminoquinoline salts, a preparation containing amodiaquine base may be found more acceptable to young children.

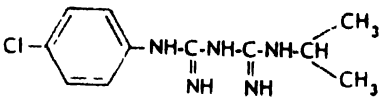
Salts in common use and physical data

Chloroquine diphosphate, sulfate and dihydrochloride; amodiaquine dihydrochloride dihydrate. See Annex 3.

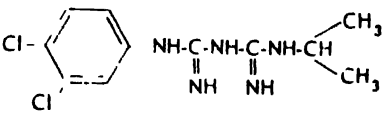
Proguanil and proguanil analogues

Structures:

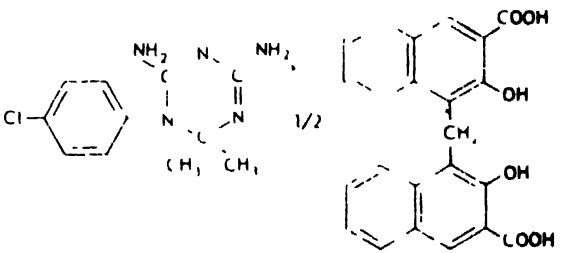
Proguanil
1-(*p*-chlorophenyl)-5-isopropylbiguanide



Chlorproguanil
1-(3,4-dichlorophenyl)-5-isopropylbiguanide



Cycloguanil embonate
4,6-diamino-1-(*p*-chlorophenyl)-1,2-dihydro-2, 2-dimethyl-*s*-triazine with 4,4 -methylene-bis(3-hydroxy-2-naphthoic acid) (2 1)



Spectrum of activity in human malaria

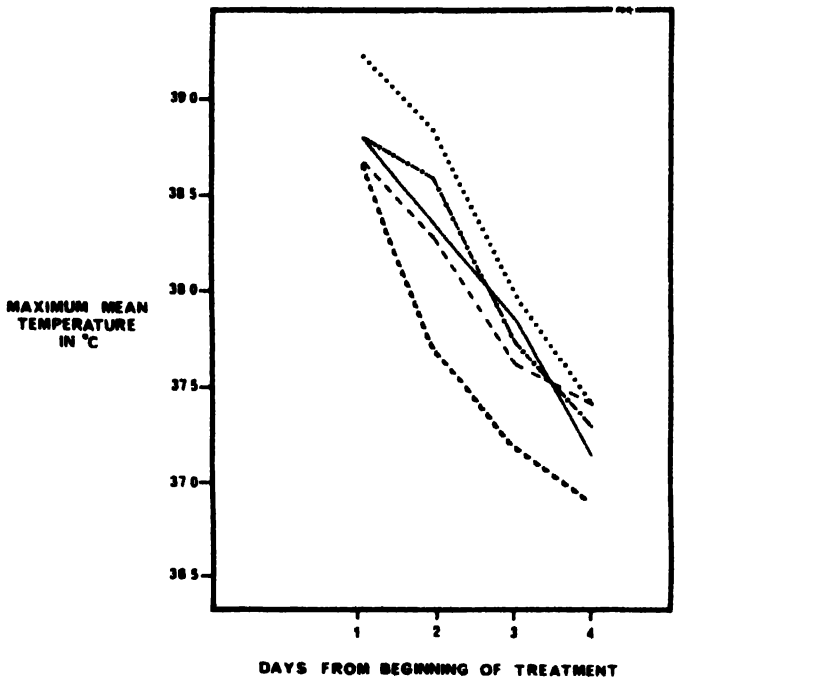
(1) Sporozoites: Probably inactive.

(2) Primary exoerythrocytic stages: Proguanil and chlorproguanil are highly active against the primary exoerythrocytic forms of *P. falciparum* and proguanil has a fleeting inhibitory action on those of *P. vivax*; its action on the primary exoerythrocytic forms of *P. malariae* is unknown. They are thus valuable drugs for *causal prophylaxis* in falciparum malaria.

(3) Asexual blood stages: Active against the asexual blood forms of all species of human malaria parasite. They achieve *clinical cure* in all forms of malaria and *radical cure* in most falciparum infections; but the clinical response is slow and their use for the treatment of an acute malarial attack is not recommended. Proguanil is a good suppressive of all forms of malaria, often with *suppressive cure* in falciparum infection (Fig. 15).

(4) Gametocytes: Proguanil and chlorproguanil have little apparent effect on the production, numbers or morphology of the gametocytes of *P. falciparum*, but in appropriate doses inhibit the later development of sporogonic forms in the mosquito. Mosquitos fed on gametocyte carriers receiving therapeutic doses do not become infective; this inhibitory effect on

Fig 15 Fever in 913 cases of acute falciparum malaria treated in Malaya with proguanil or quinine[1]



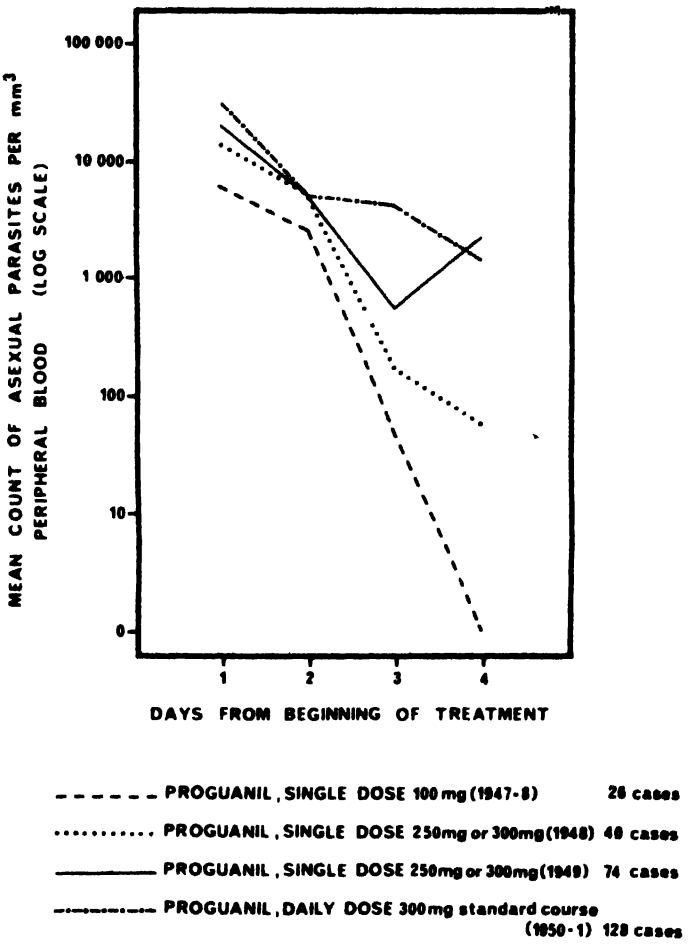
[1] Data from Malaria Research Division, Institute for Medical Research, Federation of Malaya, 1946 1951

sporogony persists for varying periods after the last dose, depending on the total quantity administered. Sporogony in *P. vivax* is similarly affected. Proguanil and chlorproguanil are thus valuable drugs for *sporontocidal prophylaxis*.

(5) Latent exoerythrocytic stages: Probably inactive, hence not effective for the radical cure of vivax malaria.

(6) General: The outstanding virtues of proguanil are its extremely low toxicity, wide range of action and relatively low cost. It is highly active as a causal prophylactic in falciparum malaria, is a good general suppressive and has a marked inhibitory effect on the transmission of malaria by mosquitos,

Fig 16 Parasitaemia in 268 cases of acute falciparum malaria treated in Malaya with proguanil, illustrating a developing resistance in the schizogonic blood forms during the period 1947–1951[1]



[1] Data from Malaria Research Division, Institute for Medical Research, Federation of Malaya, 1946–1951

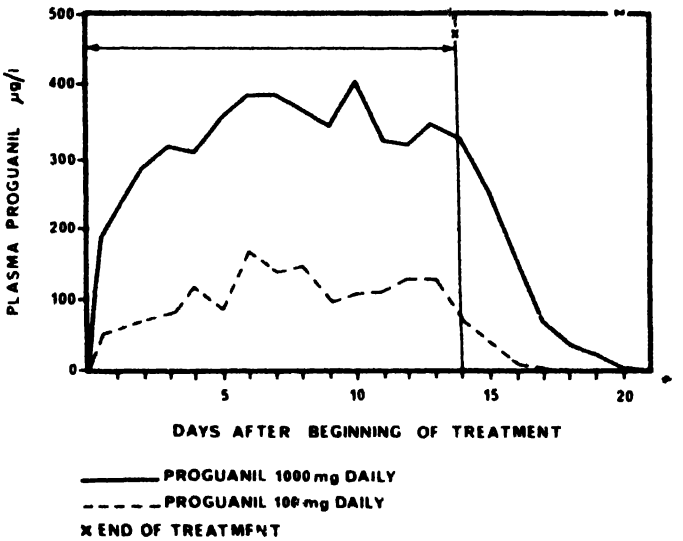
but is not sufficiently rapid in action for the treatment of the acute attack in nonimmune subjects. Its greatest drawback, a tendency to provoke resistance (Fig. 16), is discussed in Chapter 5.

Pharmacokinetics

Absorption is rapid; elimination is fairly slow and mainly in the urine, in which the drug may be detected for several days after the last dose. About

40 % is excreted in the urine and faeces, some of the remainder is converted into an active metabolite Peak concentrations in plasma are attained about 4 hours after oral administration The concentration in the red cells is 4–8 times greater than in the plasma Plasma levels fall below the level of accurate estimation within a week, even after heavy and prolonged dosage (Fig 17)

Fig 17 Concentration of proguanil (group means) in plasma during oral treatment with 100 mg and 1000 mg daily[1]



[1] After Adams A R D et al (1945) *Annals of tropical medicine and parasitology* **39** 225

Proguanil acts after conversion to a triazine metabolite, cycloguanil, by binding to an enzyme required by the malaria parasite, dihydrofolate reductase The related antimalarial compounds chlorproguanil and pyrimethamine have a similar mode of action These compounds bind also to human dihydrofolate reductase, but much less than they do to the parasite enzyme The effect of this action is to prevent the completion of schizogony This is seen in the asexual blood stages as an arrest of maturation of the developing schizonts and an accumulation of large, abnormal-looking trophozoites Proguanil does not produce the pigment clumping that characterizes chloroquine and other 4-aminoquinolines

The mean active plasma levels of proguanil in man have been reported to be between 10 and 20 µg l against *P vivax* and more than 100 µg/l against the Costa strain of *P falciparum* Against a drug-sensitive African strain of *P falciparum* the metabolite cycloguanil is fully active at 5 µg/l *in vitro* However, 250 µg/l are required against a drug-resistant strain from south-east Asia (see Table 3)

TABLE 3 *IN VITRO* ACTIVITY OF ANTIMALARIALS AGAINST DRUG SENSITIVE AND DRUG-RESISTANT STRAINS OF *P FALCIPARUM* [1]

Drug	Strain of *P falciparum*	Concentration in μg salt per litre blood											
		2500	1000	500	250	100	50	25	10	5	2.5	1.0	0.5	0
Chloroquine diphosphate	Viet Nam (Marks)	+ + +	+	0										
	Malaya (Camp)		+ + +	+ +	+	0								
	Uganda I				+ + +	+	0							
Amodiaquine dihydrochloride	Viet Nam (Marks)					+ + +								
	Uganda I						+ + +							
Amopyroquine hydrochloride	Viet Nam (Marks)					+ + +								
	Uganda I						+ + +							
Pyrimethamine isothionate	Viet Nam (Marks)	0												
	Malaya (Camp)		+ + +		+ +	+	0		+ + +	+ +	+	0		
	Uganda I													
Cycloguanil hydrochloride	Viet Nam (Marks)				+ + +	+ +	+	0						
	Malaya (Camp)					+ + +	+ +	+		0				
	Uganda I									+ + +		+ +	+	

Note + + + over 90 % of parasites affected by drug
+ + 50 90% of parasites affected by drug
+ less than 50% of parasites affected by drug
0 no drug effect (as control)
Strain Viet Nam (Marks) highly resistant to chloroquine and pyrimethamine strain Malaya (Camp) resistant to pyrimethamine strain Uganda 1 sensitive to chloroquine and pyrimethamine

[1] From WHO Technical Report Series No 529 1973 and Schmidt et al (1977) based on data from Dr K H Rieckmann

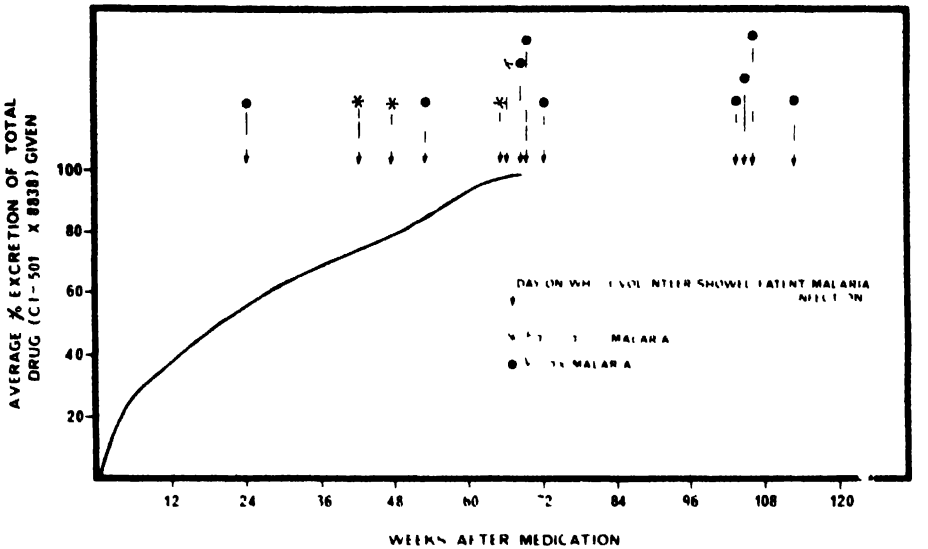
Chlorproguanil probably also acts after conversion to a triazine metabolite. It is retained in the human body much longer than proguanil. Whereas the latter must be taken daily for *causal prophylaxis* or suppression, chlorproguanil can be taken in a single weekly dose. As its triazine metabolite is, like cycloguanil, very rapidly excreted, chlorproguanil is probably tissue-(? protein-) bound, and only converted to the active metabolite after release from the binding site(s).

Cycloguanil has been administered in a poorly soluble salt, the embonate, as a repository drug. In this form it produces very prolonged *suppression*, and probably *causal prophylaxis*, of sporozoite-induced vivax and falciparum malaria (Fig. 18 and 19). Because of the likelihood that cycloguanil embonate used alone will rapidly lead to the emergence of drug-resistant malaria parasites, it is only used in combination with a sulfone, acedapsone (see p. 91).

Tolerance and toxicity

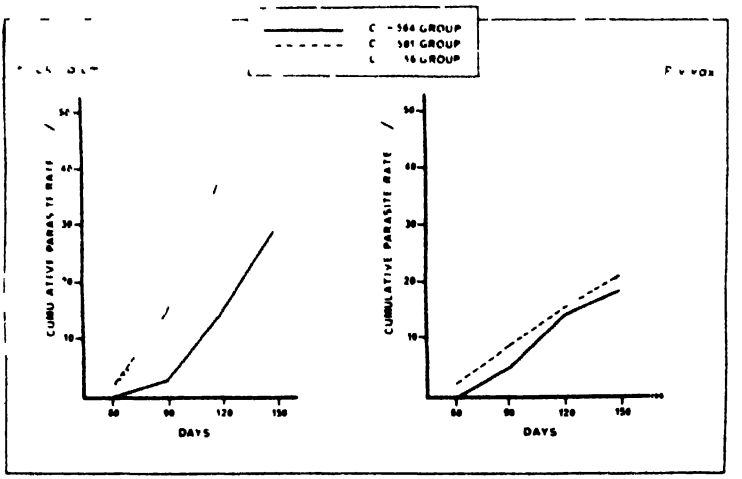
At the prophylactic dosage the toxicity of proguanil is very low; abdominal discomfort, loss of appetite, vomiting, and diarrhoea may be caused by single doses of the order of 1000 mg daily but this drug is no longer used for treatment.

Fig 18 Average urinary excretion in 6 volunteers and antimalarial activity in 13 volunteers given cycloguanil embonate (CI-501 X 8838) as a single intramuscular injection of 350 mg or at 5 mg kg body weight[1]



[1] Each volunteer was challenged one or more times either with Chesson vivax or with the SR strain of falciparum malaria by the bites of infected mosquitos
From Contacos P G et al (1966) *American journal of tropical medicine and hygiene* **15** 281

Fig 19 Comparative response of *P falciparum* and *P vivax* to repository drugs in New Guinea[1]



[1] Cumulative parasite rates by species, from 60 to 150 days after treatment of 3 groups with cycloguanil embonate (CI-501), DADDS (CI-556) or a 1 1 mixture of these (CI-564) From Rieckmann, K H (1967) *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **51** 457

Contraindications

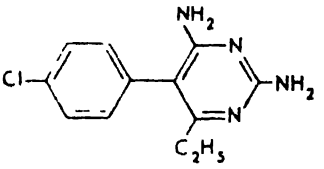
No contraindications are known other than the presence in the area of strains resistant either to proguanil or pyrimethamine (see Chapter 5). Chloroquine-resistant strains of *P. falciparum* may also be resistant to proguanil and pyrimethamine.

Salts in common use

Proguanil: hydrochloride; acetate; lactate.
Chlorproguanil: hydrochloride.
Cycloguanil: embonate.
See Annex 3.

Pyrimethamine

Structure:



Pyrimethamine
2 4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine

Spectrum of activity in human malaria

(1) Sporozoites: Probably inactive.

(2) Primary exoerythrocytic stages: It is believed that pyrimethamine has an effect against these forms, but the extent of such action has not yet been defined.

(3) Asexual blood stages: Active against these forms in all types of malaria, producing *clinical cure* in all and *radical cure* in most cases of falciparum infection. Its action is, however, slow, and it is therefore not recommended for treatment of the acute attack. Dose for dose, pyrimethamine is one of the most powerful suppressive agents known; *suppressive cure* is achieved against *P. falciparum* and sometimes against *P. vivax*. Like proguanil, it appears to act by inhibiting nuclear division, secondary to its binding to parasite dihydrofolate reductase.

(4) Gametocytes: There is no apparent effect on the production, number or morphology of gametocytes, but the action of the drug appears to inhibit subsequent sporogony in the mosquito, resulting in a decrease of transmission of the infection within the community.

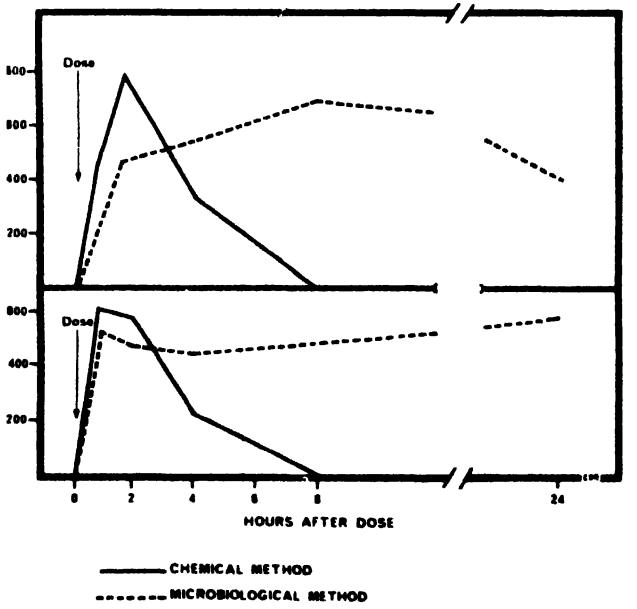
(5) Latent exoerythrocytic stages: Probably inactive. This drug is not effective for the radical treatment of vivax malaria.

(6) General. Pyrimethamine is a potent drug of extraordinary effectiveness against erythrocytic parasites Its chief characteristics are: (1) small once-weekly doses can effect complete *suppression* of quartan malaria and *suppressive cure* of falciparum malaria, (2) by inhibiting sporogony it prevents the transmission of malaria by mosquitos, (3) it is tasteless and therefore easy to administer to children, and (4) it is relatively inexpensive. Its limitations are (1) its action is too slow to warrant its use for treatment of an acute attack in a nonimmune subject, and (2) drug resistance may develop in the field, if it is administered in insufficient dosage (see Chapter 5)

Pharmacokinetics

Pyrimethamine is absorbed from the intestinal tract relatively slowly, but completely Peak concentrations following oral administration may be reached in approximately 2 hours Although the drug does not ordinarily accumulate in the plasma, it is apparently bound to the tissues and body fluids, thus having a prolonged effect Microbiological methods for determining serum levels originally proved more sensitive than chemical procedures Following a single 100-mg dose, detectable quantities were present in human serum for well over one week, and these were paralleled by continuing

Fig 20 The persistence of the antifolic activity of serum following doses of 100 mg pyrimethamine in 2 subjects comparison of chemical and microbiological assay methods[1]

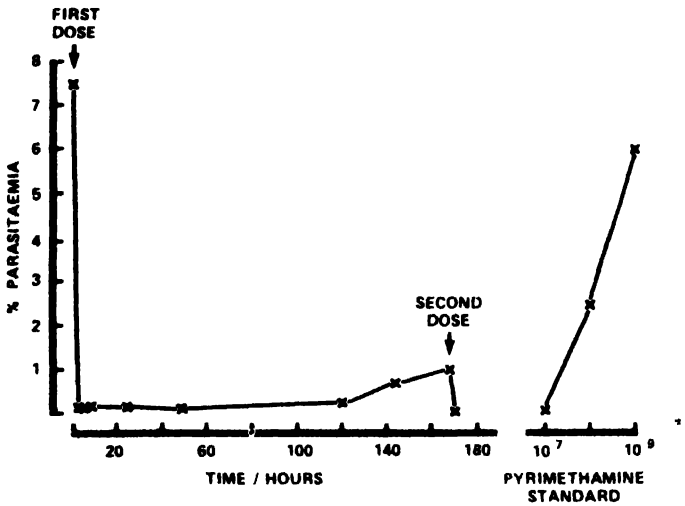


[1] From Goodwin L G (1952) *Transactions of the Royal Society of Tropical Medicine and Hygiene* **46** 485

urinary excretion, 20 to 30 % of the dose being excreted over a period of 40 days (Fig 20)

More recently, using a chromatographic technique, other workers have shown that pyrimethamine persists in the plasma and continues to be excreted in the urine for more than 14 days following the administration of a single oral dose of 25 mg Recent bioassay studies indicate that the plasma level of pyrimethamine required to inhibit blood schizogony of drug-sensitive *P. falciparum* is between 10 and 100 μg/l (Fig 21) Following a single 25-mg dose for prophylaxis this level is maintained for one week *In vitro* studies with various strains of *P falciparum* suggest that, while a level of 10 μg/l of plasma may be fully effective, a level of 1000 μg/l or more may be required against drug-resistant strains (see Table 3)

Fig 21 Plasma pyrimethamine concentrations in 6 human volunteers given 25 mg once weekly[1]



[1] Pooled plasma samples diluted to 50% of normal blood concentration were incubated with a culture of pyrimethamine sensitive *P falciparum* for 48 hours The supernatant was then replaced by RPM 1640 culture medium with 10% normal plasma and incubated for a further 48 hours At the end of this time parasitaemia levels were estimated from stained preparations and compared with those in cultures exposed under the same conditions to standard drug concentrations Unpublished data provided by Dr W H G Richards

Tolerance and toxicity

At the recommended dosage the toxicity is very low, the long-term administration of 25 mg daily (e g for the treatment of toxoplasmosis) may result in a megaloblastic type of anaemia, but remission is rapid when the drug is discontinued The effect may be countered by administering folinic acid

As pyrimethamine base is tasteless it must be kept well out of the reach of children. A number of cases of acute poisoning have been reported in toddlers who have consumed large numbers of tablets to which they have had easy access.

Contraindications

Although some authorities recommend that pyrimethamine should not be administered during pregnancy because teratogenic effects have been reported in certain animal experiments, congenital abnormalities associated with the use of pyrimethamine in the recommended antimalarial prophylactic dosage have never been recorded, in spite of its extensive use in many endemic countries for more than two decades. It now appears that pyrimethamine can safely be taken during pregnancy, and that the risk of malaria to the fetus in an unprotected mother is greater than any theoretical hazard of using pyrimethamine for prophylaxis. Pyrimethamine is contraindicated in areas where parasite strains resistant to this drug or to proguanil are well established (see Chapter 5). Chloroquine-resistant strains of *P. falciparum* are frequently resistant to proguanil and to pyrimethamine.

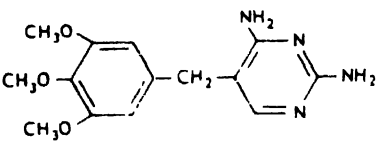
In individuals with sickle-cell trait or sickle-cell anaemia, prophylaxis with pyrimethamine or another antimalarial may be of positive benefit. If pyrimethamine is selected, however, it is advisable to give a folinic acid supplement; while preventing the depletion of folic acid in the patient, folinic acid does not reverse the antimalarial action of the drug.

Salts in common use

Pyrimethamine is prescribed as base, not as a salt (see Annex 3).

Trimethoprim

Structure:



Trimethoprim
2,4-diamino-5-(3',4',5'-trimethoxybenzyl)pyrimidine

Spectrum of activity in human malaria

(1) Sporozoites:
(2) Primary exoerythrocytic stages:
(3) Asexual blood stages:
(4) Gametocytes:
(5) Latent exoerythrocytic stages:

} Probably the same as pyrimethamine, but so far only the action against asexual blood stages has been studied critically.

(6) General: Trimethoprim is an inhibitor of dihydrofolate reductase, but has a much lower affinity for this enzyme in malaria parasites than

pyrimethamine or the proguanil metabolite cycloguanil. It has a relatively high affinity for the dihydrofolate reductase of bacteria and is therefore used in combination with a sulfonamide of correspondingly short half-life (sulfamethoxazole) in the treatment of certain antibiotic-resistant bacterial infections, against which it is highly effective.

Pharmacokinetics

After a single oral dose peak drug levels were attained within 2 - 3 hours, 9.5 mg/l being reached after a single dose of 800–1200 mg in 6 subjects. In those receiving the lower dose the biological half-life of trimethoprim in the serum was 16.5 hours and in those receiving the higher dose it was 21.3 hours. The drug is excreted mainly in the urine. Table 4 shows the comparative inhibitory activity of trimethoprim, pyrimethamine and cycloguanil on dihydrofolate reductase extracted from *Escherichia coli*, mouse erythrocytes or *P. berghei*.

TABLE 4 INHIBITION OF DIHYDROFOLATE REDUCTASES BY ANTIMALARIALS *IN VITRO*[1]

Compound	Concentration to produce 50% inhibition of enzyme from various sources (× 10 ^{8}mol/l)		
	Mouse red cells	*E. coli*	*P. berghei*
Pyrimethamine	100	250	~ 0.05
Cycloguanil	160	'	0.36
Trimethoprim	> 100 000	0.5	7.0

[1] After Ferone et al., 1969

Tolerance and toxicity

Doses of 2 g daily may cause anorexia, nausea or vomiting, which can be alleviated by giving trimethoprim in divided doses. A dose of 2 g is higher than that used for the prevention or treatment of malaria; it is otherwise well tolerated. Prolonged administration may be associated with a slight leukopenia of the type generally associated with the long-term administration of dihydrofolate reductase inhibitors. High dosages in experimental animals produce teratogenic effects, which can be reduced by administering folinic acid during gestation.

Contraindications

See comments on pyrimethamine. Since experience indicates the safety of pyrimethamine during pregnancy, pyrimethamine should be advised rather than trimethoprim for women of child-bearing age or those known to be pregnant.

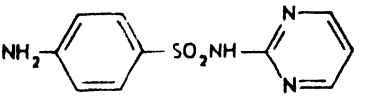
Salts in common use

Trimethoprim is employed as base. It is not advised for use alone but in combination with sulfalene (p. 132).

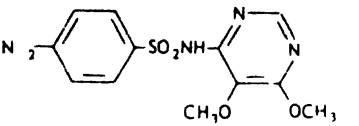
Sulfonamides and Sulfones

Structures

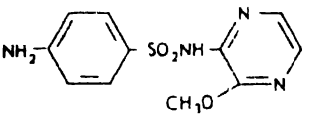
Sulfadiazine
N-2-pyrimidinylsulfanilamide



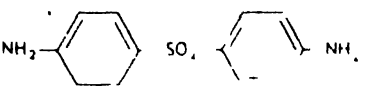
Sulfadoxine
N'-(5,6-dimethoxy-4-pyrimidinyl) sulfanilamide



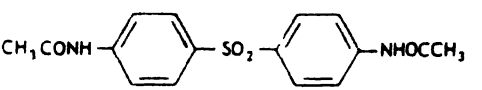
Sulfalene
N-(3 methoxy 2 pyrazinyl) sulfanilamide



Dapsone
4,4-diaminodiphenylsulfone



Acedapsone
4,4 diacetyldiaminodiphenylsulfone



Spectrum of activity in human malaria

(1) Sporozoites Inactive

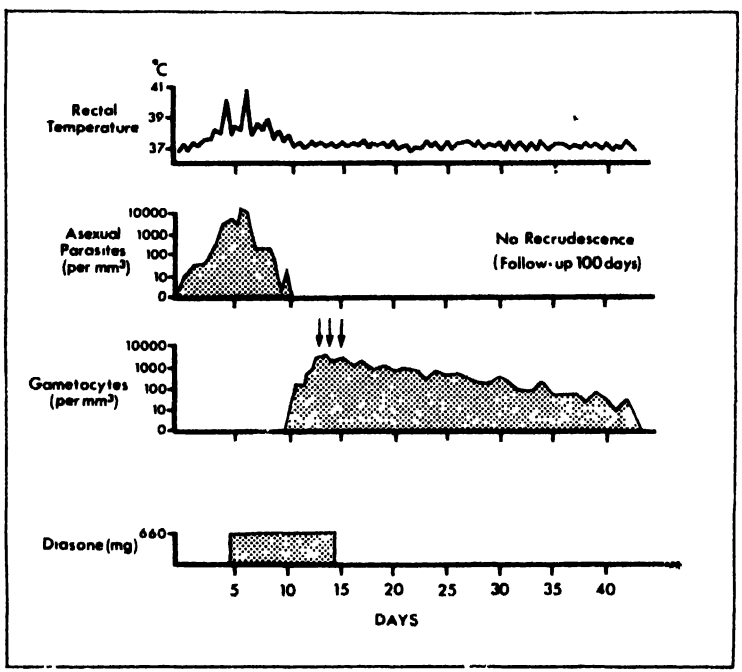
(2) Primary exoerythrocytic stages Probably inactive against all types of malaria

(3) Asexual blood stages Sulfonamides and sulfones are highly effective against the asexual blood forms of *P falciparum* but less effective against those of the other species They produce *clinical cure* of falciparum malaria, but even against this infection their action is too slow for them to be used

alone. They are also effective *suppressive* agents, but should not be used for this purpose alone because of the rapidity with which drug resistance can develop. They produce morphological changes in the developing schizonts rather similar to those noted with proguanil.

(4) Gametocytes: When administered alone to patients with falciparum malaria they appear to cause waves of increased gametocyte production (Fig. 22). However, these gametocytes may not be infective to mosquitos. In experimental rodent malaria sulfonamides exert a *sporontocidal* action against the parasites in the mosquito.

Fig. 22. Action of a DDS analogue alone against malayan (Camp) strain of *P falciparum* in a nonimmune subject[1]



[1] Administration of 660 mg daily for 10 days effected *gradual* clearance of asexual parasitaemia and radical cure, but heavy gametocytaemia developed. Mosquitos fed at the times indicated by the arrows did not become infected.

From: Powell, R. D. et al (1966) *Proceedings of the third international pharmacology meeting, São Paulo, 1966*, vol. 1, p. 39.

(5) Latent exoerythrocytic stages: Inactive. They do not produce a *radical* or *suppressive cure* of vivax malaria.

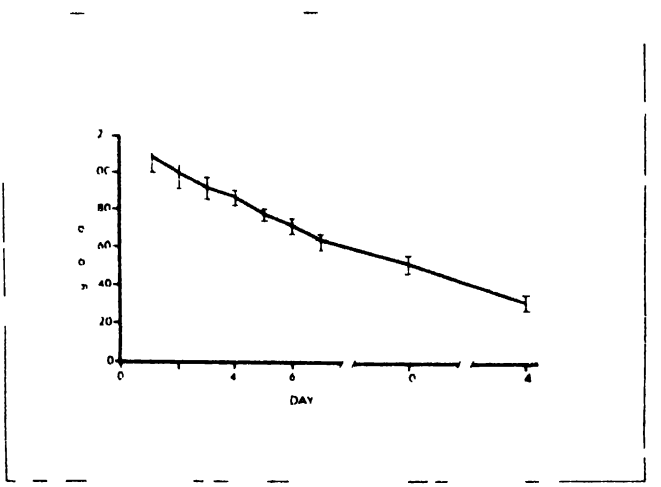
(6) General: Malaria parasites, like many bacteria, are unable to utilize preformed folic acid and require para-aminobenzoic acid as a substrate in

order to synthesize it Sulfonamides and sulfones act as competitive antagonists of this substrate Pyrimethamine and proguanil (and related compounds) inhibit a later step in the folate synthetic pathway, that mediated by the enzyme dihydrofolate reductase When administered together with those compounds, sulfonamides or sulfones may potentiate the action of dihydrofolate reductase inhibitors This potentiation may be of such a degree that the combination can be effective against strains of microorganisms that are resistant to either component used alone However, malaria parasites can rapidly develop resistance to compounds of either group when they are employed alone The sulfonamides and sulfones possess the general disadvantages of this group of compounds as regards toxicity (see below)

Pharmacokinetics

There is a considerable variation in the rate of absorption and excretion of different sulfonamides following oral administration, depending upon a number of factors including the degree to which they are bound to protein and metabolized They are excreted mainly in the urine as metabolites The 2 compounds currently in use as antimalarial agents are sulfadoxine (Fig 23) and sulfalene, both of which have a prolonged half-life in man

Fig 23 Plasma levels of sulfadoxine in patients with falciparum malaria[1]



[1] Free sulfonamide levels (concentration mean ± S E) following administration of a single oral dose of 1 g sulfadoxine to 15 patients together with pyrimethamine and quinine The plasma half life of approximately 200 hours reflects the slow elimination of the drug in urine

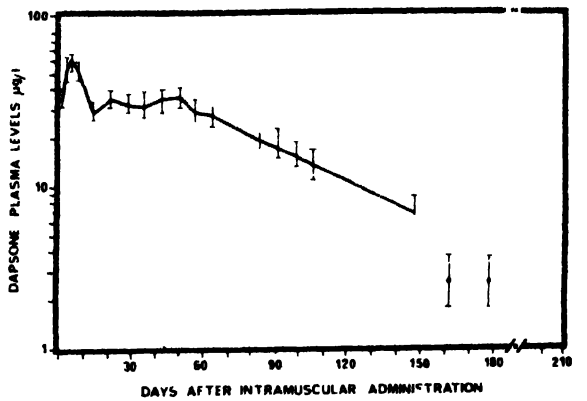
From Brooks M H et al (1969) *Clinical pharmacology and therapeutics* 10 85

The half-life of sulfadoxine is estimated to range from 100 to 200 hours. Only a small proportion is metabolized, about 5 % to the 4-acetyl derivative and 2–3 % to the glucuronide Another sulfonamide with a prolonged half-life in man (65 hours) is sulfalene Both compounds are used in fixed combinations with pyrimethamine (see Chapter 6), which also has a long half-life

The sulfone, dapsone, is well absorbed by mouth, peak serum levels being found within 3 6 hours The mean half-life of dapsone following a single oral dose is approximately 28 hours The diacetyl derivative of dapsone, acedapsone, is poorly soluble When injected intramuscularly in man, a 300-mg dose was found to have a half-life averaging 42 6 days Acedapsone is slowly dissolved from the injection site and deacetylated to the active monoacetylate and parent sulfone, both of which are active

Recent studies have shown that there is an important genetic factor governing the rate at which different individuals acetylate sulfonamides, "fast acetylators" reducing the effectiveness of these compounds more rapidly than "slow acetylators" This factor, rather than the development of drug-resistant parasites, may account for some apparent failures with these drugs in falciparum malaria The hypothesis, however, is disputed by some authorities Since sulfones are metabolized in different ways, this factor may not come into play with dapsone or acedapsone (Fig 24)

Fig 24 Fall in plasma levels following intramuscular injection of a single 300 mg dose of acedapsone in man[1]



[1] Heavy line indicates mean values ± S E calculated for 5 men on days 0 to 57, 4 men on days 64 to 232
Redrawn from Glazko, A J et al (1968) *American journal of tropical medicine and hygiene*, 17 465

Tolerance and toxicity

Sulfadoxine and sulfalene administered in the correct dosage are well tolerated as a rule but, like all sulfonamides, may produce side effects in certain individuals Occasional skin reactions in the form of urticaria occur Rarely, a more serious reaction of the Stevens–Johnson type has been reported but only following gross overdosage Adverse effects on the haematopoetic system have been generally few and limited to a slight depression of the granulocyte count, though some more severe effects have also been observed including agranulocytosis It is important to remember that these compounds have a prolonged half-life and that they are administered in much smaller doses than the older, rapidly excreted, sulfonamides

Both sulfonamides and sulfones can precipitate haemolysis in individuals who are G6PD-deficient They can also produce methaemoglobinaemia in those with hereditary NADH methaemoglobinaemia reductase deficiency Since primaquine can also precipitate these effects, special care must be taken if sulfonamides or dapsone are given sequentially or concurrently with primaquine (see Fig 12, p 64)

Contraindications

Sulfonamides and sulfones are contraindicated in individuals with a history of hypersensitivity and in premature or newborn infants during the first month of life Although there are no reports of teratogenic effects of sulfadoxine or sulfalene, it is generally advised that the administration of these compounds should be avoided during the first trimester of pregnancy (see also p 80 concerning pyrimethamine) Sulfones appear to be free of this potential risk

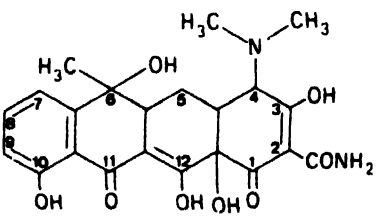
Salts in common use

Sulfonamides and sulfones are administered as bases (see Annex 3)

Tetracyclines[1]

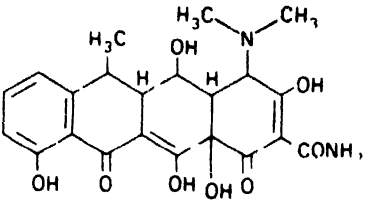
Structures

Tetracycline
4-dimethylamino 1 4 4a 5,5a,6 11 12a octahydro 3 6 10 12,12a pentahydroxy 6 methyl 1 11 dioxo-2 naphthacenecarboxamide



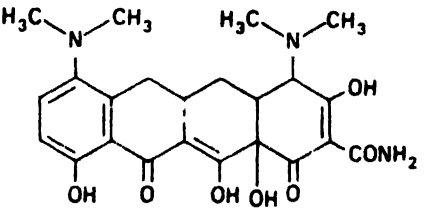
Doxycycline

4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide



Minocycline

4,7-bis(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,10,12,12a-tetrahydroxy-1,11-dioxo-2-naphthacenecarboxamide



Spectrum of activity in human malaria

(1) Sporozoites. Unknown.

(2) Primary exoerythrocytic forms: Tetracyclines are active against the primary exoerythrocytic forms of *P. falciparum* even when administration is started as late as 4 days after the infective bite. (However, the use of antibiotics for the *causal prophylaxis* of malaria is *not* advised on the general principle that such compounds should be reserved for treatment in the special conditions indicated at (6) below.) The action of the tetracyclines against the primary exoerythrocytic stages of other species of human malaria has been inadequately studied. Tetracycline has been shown to have an action on *P. vivax* in the chimpanzee and *P. cynomolgi* in the rhesus monkey.

(3) Asexual blood stages: The tetracyclines exert a blood schizontocidal action against *P. falciparum*, including infections with strains that are resistant to the 4-aminoquinolines and dihydrofolate reductase inhibitors. They can be used for the treatment of falciparum infections resistant to these drugs, but, as the clearance of fever and parasitaemia is rather slow, they should always be used in conjunction with quinine. *The value of tetracyclines* as blood schizontocides against other species of human malaria has not been adequately documented.

(4) Gametocytes: Tetracyclines appear to have no gametocytocidal activity against *P. falciparum*. No data are available on other species.

(5) Latent exoerythrocytic parasites: Tetracyclines apparently do not produce a *radical cure* of vivax malaria.

(6) General: Tetracyclines are potent antibacterial agents and their use should be restricted, as far as possible, to the treatment of bacterial infections [illegible] to organisms that can be demonstrated to be tetracycline-

sensitive. It is quite unnecessary to treat any but falciparum malaria with antibiotics, and even in this case tetracyclines should be reserved for use only against strains that are known to be resistant to standard antimalarials such as chloroquine, when (i) no other remedy is available (e.g. quinine or sulfadoxine-pyrimethamine), or (ii) the patient is known to be hypersensitive to sulfonamides, or (iii) the infection has not adequately responded to treatment with sulfonamide/pyrimethamine combinations.

Tetracyclines interfere with protein synthesis in bacteria probably by preventing the formation of peptide linkages. Their prime mode of action against malaria parasites has not been determined.

Pharmacology

Most of the tetracyclines are crystalline substances, amphoteric and of low solubility. The hydrochlorides are more soluble and are the compounds chiefly used in therapeutics. The stability of the compounds varies considerably. Their absorption takes place from all levels of the alimentary canal, but only a proportion of the compound is absorbed. The blood curve is a plateau with a slow rise for 3 - 4 hours and a still slower fall. Tetracyclines are freely excreted in both the bile and the urine. Urinary excretion accounts for about 20 % of an average oral dose. Suitable solutions can be administered by slow intravenous drip.

The pharmacokinetics of tetracyclines depend upon the individual compound used. Reference should be made to standard works for details (Garrod et al., 1973; Goodman & Gilman, 1975).

Tolerance and toxicity

Tetracycline in the oral dosage occasionally used for the treatment of malaria (e.g. 250 mg 4 times a day for up to 7 days) may cause nausea, vomiting and diarrhoea. The newer derivatives such as doxycycline and minocycline are given in smaller doses and are generally better tolerated.

Tetracyclines sometimes have more serious adverse effects on the skin, mucous membranes and gastrointestinal tract. Tetracycline complexes may also be deposited in growing teeth and bones, consequently they should be avoided in women after the fourth month of pregnancy and in infants or young children.

Other contraindications to the use of tetracyclines and related antibiotics for the treatment of malaria are mentioned in Chapter 6.

Potentiating Combinations of Antimalarial Compounds

General

Reference has been made to the potentiating action against malaria parasites obtained by administering a sulfonamide or sulfone together with

an inhibitor of dihydrofolate reductase This effect can readily be demonstrated in experimental animal models (Fig 25).

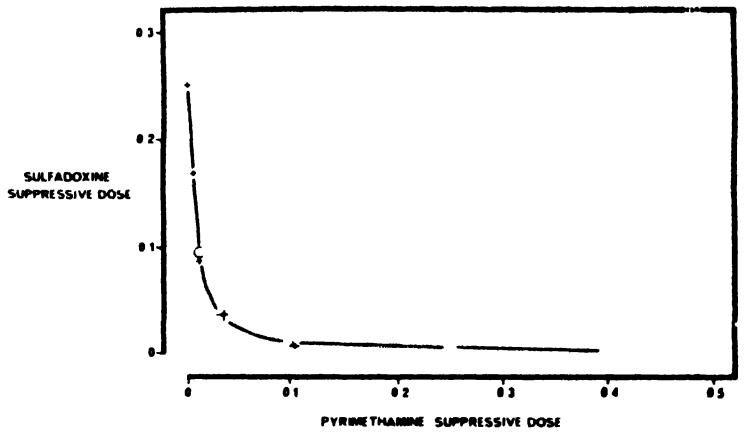
The potentiating action can be of such a high degree that the combination is effective against strains of *Plasmodium* that are resistant to each of the individual components Two further benefits of such combinations are that (i) a reduced dose of each component can be administered and (ii) the continued use of the combination in an individual or a community reduces the chances of resistance developing to the component drugs in the parasite population The combination may also be more effective than the individual components against certain stages (e g the pre-erythrocytic schizonts of *P vivax*) (Fig 26)

Selection of combinations

It is important that the half-lives of the individual components of potentiating combinations should be as well matched as possible, and that they are given in an optimal proportion It must be admitted that neither condition has been determined critically with most of the combinations summarized in Table 5

When the half-lives of the 2 components are not reasonably close to each other, the frequency with which the combination is administered prophylacti-

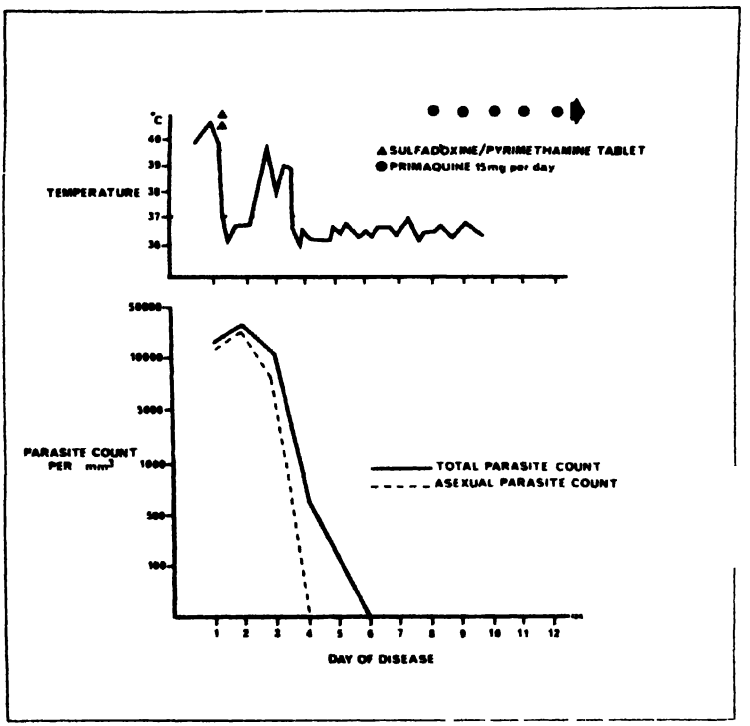
Fig 25 Potentiation of the suppressive action of sulfadoxine and pyrimethamine against *P berghei* in mice[1]



[1] Ordinates and abcissae show the daily doses in mg kg given for 4 successive days The figure is plotted from calculated values of the 90% suppressive dose (ED_{90}) obtained when a fixed dose of sulfadoxine was given with varied doses of pyrimethamine (+), or a fixed dose of pyrimethamine was administered with varied doses of sulfadoxine (o) A simple additive effect would be present if all the points fell on or near the dotted line The curve well below this line indicates a marked degree of drug potentiation

From Peters, W (1968) *Annals of tropical medicine and parasitology*, **62**, 488

Fig 26 Response of *P vivax* parasitaemia in a patient infected in Sumatra[1]



[1] Administration of a combination of 1 g sulfadoxine with 50 mg pyrimethamine in a single dose Note that the asexual parasite count increased for 24 hours after drug administration Defective late-stage trophozoites or preschizonts were observed after 48 hours From Ebisawa I et al (1974) *Japanese journal of experimental medicine* **44** 151

cally should be adjusted according to the half-life of the component most rapidly eliminated, bearing in mind that, once the concentration of the latter has fallen below the effective level, prophylaxis will depend entirely upon the second component

Tolerance and toxicity

See the sections on individual components In the doses recommended for malaria prophylaxis or therapy the combinations of pyrimethamine with sulfadoxine, sulfalene or dapsone have been remarkably well tolerated Occasional side effects of the types to be anticipated with the individual components have been noted, such as mild headache, nausea and occasional vomiting Allergic skin reactions have been recorded rarely Prolonged use of

TABLE 5. ESTIMATED HALF-LIVES OF COMPOUNDS USED IN POTENTIATING COMBINATIONS AND PROPORTIONS OF COMPONENTS

Component	Half life	Proportion
Pyrimethamine	96 192 hours	1
+		
sulfadoxine	100 200 hours	20
Pyrimethamine	96 192 hours	1
+		
sulfalene	65 hours	20
Pyrimethamine	96 192 hours	1
+		
dapsone	17 33 hours	8
Trimethoprim	< 16 5 hours	2
+		
sulfalene	65 hours	1
Cycloguanil embonate	~ 100 140 days	1
+		
acedapsone	~ 43 days	1
Proguanil	24 hours	8
+		
dapsone	17 33 hours	1

these combinations for prophylaxis has sometimes been associated with a slight fall in the leukocyte count, but it has reverted to normal on the administration of folinic acid or on withdrawal of the drugs. Haematocrit values have actually increased during some field studies, in part probably because of a reduced incidence of malaria parasitaemia and its associated haemolysis

Contraindications

See individual components

Salts in common use and physical data

See Annex 3

CHAPTER 4

NEW ANTIMALARIAL DRUGS UNDER DEVELOPMENT

General trends

New antimalarial drug development declined sharply following the introduction of chloroquine for both the treatment and the prevention of all types of malaria and of primaquine for the radical cure of relapsing malaria However, the discovery in the early 1960s of strains of *P falciparum* that were resistant to chloroquine stimulated renewed interest in antimalarial chemotherapy and new drug research was undertaken by several government and private agencies In recent years, the UNDP World Bank WHO Special Programme for Research and Training in Tropical Disease has attempted to provide a forum for the exchange of information between these groups In addition, the Special Programme is studying the improvement of existing drugs for potential mass drug administration and the further development of new drugs as they become known

Much of the research on new antimalarial compounds has been summarized in the report of the WHO Scientific Group on Chemotherapy of Malaria (1973) and in the reports of two meetings on Chemotherapy of Malaria organized by the UNDP/World Bank WHO Special Programme for Research and Training in Tropical Diseases [1] [2] Only a few of the studies need to be mentioned here and the interested reader should refer to the original reports

It appears that the most promising developments in the field of new antimalarial compounds are in the large-scale research programme carried out under the auspices of the Government of the USA Since 1963, when the US Army Research Program in Malaria was launched, more than 250 000 separate chemical entities have been examined for antimalarial activity About 3 % or 7 500 showed significant activity in animal primary test systems

The methods employed in this vast research programme are of interest The compounds are selected initially by the Division of Experimental Thera-

[1] Report of the first meeting of the task force on chemotherapy of malaria (unpublished WHO document TDR CM 76 1)

[2] Report of the second scientific working group on chemotherapy of malaria (unpublished WHO document TDR CHEMAL-SWG (2) 78 3)

peutics of the Walter Reed Army Institute of Research on the basis of the results of antimalarial efficacy studies in various primary and secondary test systems, particular attention being paid to activity against resistant strains of human plasmodia in the owl monkey (*Aotus trivirgatus*) and *in vitro*. When possible, several structurally similar compounds are evaluated concurrently and the most active analogues are selected. A batch of the selected compound is then prepared and its composition, purity, and stability are evaluated by independent assay.

Acute and subacute tolerance studies are performed in at least two animal species. In some cases, the results of routine clinical studies and experience with similar compounds indicate a need for special pharmacological investigations, including testing for phototoxicity or cardiovascular toxicity. The preclinical data are then evaluated to assess the risk-benefit ratio of the compound. If human trials are recommended, the dosage schedule, clinical studies, and laboratory examinations are determined individually for the compound. All the data are then reviewed by four groups of scientists, one of them examining the ethical implications and ensuring that the subjects are fully informed and consent voluntarily to the trial. The results of the trials are continuously monitored. Compounds that have been shown to have a greater therapeutic activity or lower toxicity than existing drugs undergo further testing.

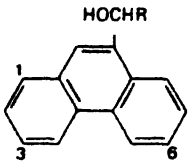
By 1979, 43 different chemical compounds and four combinations of compounds had been selected for clinical trial. Many hundreds more had shown varying degrees of activity in one or more test systems, but only the most active within each class of compounds had been selected.

Many of the compounds tested have been superseded because of evidence of intolerance or the continued discovery of even more active drugs, leading to the elimination of those of lesser activity. At present it is not possible to predict which compounds will ultimately be incorporated into the list of reliable antimalarial drugs.

This review is limited to a discussion of some of the more active chemical classes of drugs and data are presented on specific drugs only as far as they are deemed to be examples of the relevant class

In the discussion of the characteristics of several new antimalarial drugs the tables indicate the antiplasmodial efficacy of selected compounds. The abbreviation SN refers to the serial number from the *Survey of antimalarial drugs, 1941–1945*, and WR to the Walter Reed Army Institute of Research designation for the compounds selected. All structural substitutions not specified are H. Primary mouse CD_{50}s were calculated from probit transformation of dose-response antimalarial activity against *P. berghei* in mice. Owl monkey CD_{50}s were similarly calculated on antimalarial activity against *P. falciparum* in the owl monkeys. Human curative dose refers to the total dose for curative effect against *P. falciparum*. Blank spaces indicate that the drug was not tested in that system.

9-Phenanthrenemethanols



Drug	Substitutions	Primary mouse CD_{50} (mg/kg)	Owl monkey CD_{50} (mg/kg)	Human curative dose (g man)
SN 8867	R = $CH_2N[(CH_2)_8CH_3]_2$	458		
SN 9160	6 = Cl, R = $CH_2N[(CH_2)_6CH_3]_2$	> 640		
WR 33063	6 = Br, R = $CH_2N[(CH_2)_4CH_3]_2$	462	4300	9 6
WR 122455	3 = CF_3, 6 – CF_3, R = 	30 4	16 7	1 4
WR 171 669	1 = Cl, 3 = Cl, 6 = CF_3, R = $(CH_2)_2N(C_4H_9)_2$	15 0	58 2	3 0

(1) *General.* Five phenanthrenemethanols have been tested for antimalarial activity in man. The first two (SN 8867 and SN 9160) were tested in blood-induced vivax infection in volunteers during the Second World War and found to have good blood schizontocidal activity. The third (WR 33 063) was tested against naturally acquired drug-resistant falciparum malaria in Viet Nam and Thailand and found to be rapidly acting and superior to any single antimalarial drug available at that time for these resistant strains. The fourth and fifth (WR 122 455 and WR 171 669) have only been tested in volunteer subjects, but both show even greater activity against multidrug-resistant falciparum malaria.

(2) *Spectrum of activity.* The phenanthrenemethanols are effective against the erythrocytic stages of all species of malaria tested. They are ineffective against exoerythrocytic forms. *In vitro* studies of intraerythrocytic *P. falciparum* show no evidence of cross-resistance in strains resistant to the common antimalarial drugs.

(3) *Toxicity.* In general, the phenanthrenemethanols are well tolerated in man. The usual dose-limiting factor has been gastrointestinal symptoms such as cramping abdominal pain, nausea or diarrhoea. Although some of the phenanthrenemethanols tested show evidence of phototoxicity in animals, no such effect has been seen with the specific drugs tested in man. However, 3 subjects developed a skin rash with the first phenanthrenemethanol tested (SN 8867).

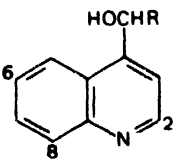
(4) *Pharmacology.* The phenanthrenemethanols appear from animal studies to be poorly absorbed or largely excreted in the bile, the greatest

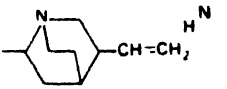
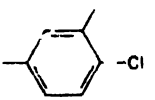
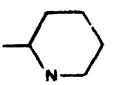
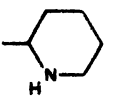
amount of orally administered drug appearing in the faeces. WR 122455 appears to have a somewhat longer half-life, with measurable plasma levels persisting for several days in animals. Kinetic studies have not been performed with these drugs in man.

(5) *Clinical results.* WR 33 063 in doses of 1.6 g daily for 6 days was uniformly effective in volunteers infected with drug-sensitive strains of *P. falciparum* and cured about 80% of volunteers with multidrug-resistant strains. Slightly greater doses cured all 13 patients who were treated with this drug for naturally acquired falciparum malaria from south-east Asia that had not been cured by multiple courses of standard drugs. Studies in both semi-immune and nonimmune patients in south-east Asia showed an average cure rate of 90% with a single course of treatment.

WR 122455 and WR 171669 have only been evaluated in volunteers with induced infections. Daily doses of 500 mg of WR 122455 or 1 g of WR 171669 for 3 days or greater were uniformly effective in curing drug-resistant falciparum malaria in the few volunteers tested with these drugs.

4-Quinolinemethanols



Drug	Substitutions	Primary mouse CD, (mg/kg)	Owl monkey CD (mg/kg)	Human curative dose (g/man)
SN 10275	6 = Cl, 8 = Cl, 2 = C_6H_5, P –	42.0	18	
quinine	6 = CH_3O, R = 	1300	297	19.5
WR 30090	6 = Cl, 8 = Cl, 2 =  R = $CH_2N[(CH_2)_3CH_3]_2$	34.7	114	4.8
mefloquine	2 = CF_3, 8 = CF_3, R = 	54.5	7.3	1.5
WR 184806	2 = CF_3, 8 = CF_3, R = $(CH_2)_2NHC(CH_3)_3$	298	38	
WR 226253	2 = CF_3, 6 = Cl, 8 = Cl, R = 	167	5.3	

(1) *General.* Excluding isomers of quinine, 14 quinolinemethanols have been evaluated for antimalarial activity in man, and other active derivatives of this class are scheduled for evaluation. Twelve of these were tested in blood-induced vivax malaria infections in volunteers during the Second World War. The most active of those tested, SN 10 275, was found to have good blood schizontocidal activity, but its clinical use was seriously limited because of phototoxicity. The 2 most recently developed, WR 30 090 and WR 142 490 (mefloquine), were tested against naturally acquired acute infections by drug-resistant *P. falciparum* in south-east Asia and found to be very active Mefloquine was curative in nearly all cases with a single oral dose. It was also found to be an outstanding suppressive prophylactic drug when administered weekly or fortnightly for drug-resistant falciparum and vivax infections.

(2) *Spectrum of activity.* The quinolinemethanols are effective against the erythrocytic stages of all species of malaria parasite. The mechanism of action is unknown. Unlike chloroquine, mefloquine, the most active drug of this series, does not bind to DNA. *In vitro* studies of intraerythrocytic *P. falciparum* show no evidence of cross-resistance when tested against strains resistant to the common antimalarial drugs. The quinolinemethanols are ineffective against exoerythrocytic forms.

(3) *Toxicity.* In man the quinolinemethanols are generally well tolerated. The usual symptoms of quinine toxicity have not been observed thus far with these more recent analogues. However, single oral doses in excess of those required to cure malaria have produced lightheadedness and occasional gastrointestinal symptoms. Although phototoxicity was a serious side effect with SN 10 275, the newer analogues have not shown clinical evidence of this adverse effect, although an occasional skin rash has been reported. Repeated high daily doses of mefloquine given to rats have apparently caused histologic abnormalities of the retina and reversible epididymal injury in males.

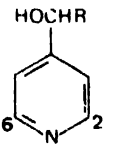
(4) *Pharmacology.* The quinolinemethanols appear to be somewhat better absorbed than the phenanthrenemethanols. As in the latter group, they appear to be excreted in the bile. The duration of action is variable, with SN 10 275 and mefloquine exhibiting a prolonged effect. The half-life of WR 30 090 is about 24 hours and that of mefloquine about 2 weeks.

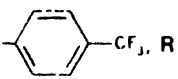
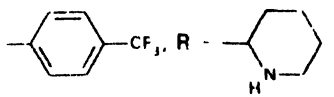
(5) *Clinical results.* WR 30 090 in doses of 0.7 g daily for 6 days was uniformly effective in volunteers infected with sensitive strains of falciparum malaria and cured about 90 % of volunteers with multidrug-resistant strains. The same dose also cured 7 out of 7 patients with naturally acquired falciparum malaria from south-east Asia who had had multiple recrudescences following standard treatment regimens. Further studies on naturally acquired acute falciparum infection in both semi-immune and nonimmune patients in south-east Asia showed an average cure rate of 90 % with a single course of treatment.

Mefloquine in single doses of 1.5 g was uniformly effective in curing volunteers with multidrug-resistant strains of falciparum malaria. Studies in semi-immune patients in Thailand showed that this dose was nearly 100 %

effective in curing naturally acquired infection with drug-resistant strains of *P. falciparum*. Used as a suppressive prophylactic, either weekly or fortnightly, smaller doses of the drug have also been shown in a large study of nearly 500 subjects to prevent attacks of both vivax and falciparum malaria.

4-Pyridinemethanols



Drug	Substitutions	Primary mouse CD_{50} (mg/kg)	Owl monkey CD_{50} (mg/kg)
WR 172435	2 & 6 –  CF_3, R – $(CH_2)_2N(C_4H_9)_2$	28 5	22 0
WR 180409	2 CF_3 6  CF_3, R –	50 0	18 6

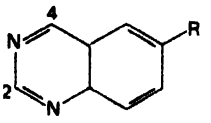
(1) *General.* No pyridinemethanols have been tested for antimalarial activity in man. However, many are active in animal malaria models and 2 have been selected for clinical trials (WR 172 435 and WR 180 409).

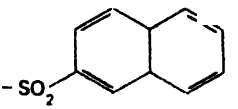
(2) *Spectrum of activity.* Animal studies have shown good evidence of activity against the erythrocytic stages of the parasites in all species of malaria tested, including highly multidrug-resistant *P. falciparum* in owl monkeys. *In vitro* studies of intraerythrocytic *P. falciparum* showed no evidence of cross resistance when tested against strains resistant to the common antimalarial drugs. The pyridinemethanols are ineffective against exoerythrocytic forms.

(3) *Toxicity.* Studies with WR 180 409 in a few volunteers have shown that the drug is well tolerated in single doses as large as 1 g. Larger single doses produced nausea, vomiting and dizziness. Additional studies are planned to determine the optimum regimen for therapeutic trials.

(4) *Pharmacology.* The pyridinemethanols appear in animal studies to be moderately well absorbed and largely excreted in the bile, the greatest amount of the orally administered drug appearing in the faeces. Both WR 172 435 and WR 180 409 appear to persist in tissues for some time and require only single oral doses to cure monkeys with multidrug-resistant falciparum malaria.

(Arylthio)quinazolines



Drug	Substitutions	Primary mouse CD_{50} (mg/kg)	Owl monkey CD_{50} (mg/kg)
WR 158122	2 = NH_2, 4 = NH_2, R = 	12 1	0 02-0 03[a]

[a] Against pyrimethamine-sensitive, pyrimethamine-resistant *P falciparum*

(1) *General.* Only a single (arylthio)quinazoline has been tested for antimalarial activity in man. This drug, WR 158 122, was extremely active in animal test systems but showed only limited activity in induced infections in volunteers.

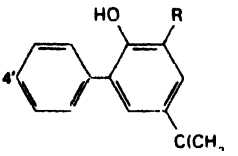
(2) *Spectrum of activity.* The (arylthio)quinazolines are effective against the erythrocytic stages of all species of malaria parasite tested and exhibit marked potentiation with the sulfonamides. The mechanism of action is by inhibition of dihydrofolic acid reductase and there is both *in vitro* and *in vivo* evidence of significant cross-resistance with pyrimethamine.

(3) *Toxicity.* The single (arylthio)quinazoline tested in man was well tolerated at the greatest dose administered, 1.3 g daily for 3 days.

(4) *Pharmacology.* WR 158 122 is probably well absorbed orally. It is tightly bound to the plasma proteins but cleared rapidly from the blood and excreted in the bile. The plasma levels, estimated by microbiological assay following single doses up to 250 mg, were very low for the first few hours and became undetectable in 24 hours. Given by parenteral injection, small amounts can protect mice for as long as 3 weeks; this suggests slow absorption from the site of injection.

(5) *Clinical results.* WR 158 122 in doses of 1 g daily for 3 days cured 1 out of 2 volunteers with drug-sensitive falciparum malaria. The drug was also administered in combination with sulfadiazine (200 mg/2 g) daily for 3 days to volunteers with the same drug-sensitive strain and all 3 were cured.

Phenylphenols



Drug	Substitutions	Primary mouse CD_{50} (mg/kg)	Owl monkey CD_{50} (mg/kg)
SN 7744	$R = CH_2N(C_2H_5)_2$	807	
WR 194 965	4' = Cl, $R = CH_2NHC(CH_3)_3$	163	16 2

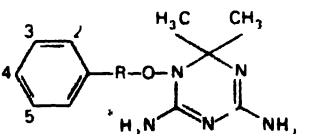
(1) *General.* Only 2 phenylphenols have been tested for antimalarial activity in man. The most promising (SN 7744) was tested against blood-induced vivax infection in volunteers during the Second World War and was found to be only slightly active. Recently, however, enhanced activity in animal test systems has been discovered by further structural modification, and further studies of antimalarial efficacy in man are planned.

(2) *Spectrum of activity.* The phenylphenols have been evaluated only for blood schizontocidal effects and in these tests showed activity against all the strains tested. Cross resistance has not been completely evaluated, but WR 194965 is very effective against the multidrug-resistant Smith strain of *P. falciparum* in owl monkeys.

(3) *Toxicity.* Studies with WR 194965 in a few volunteers have shown that the drug is well tolerated in single doses up to 1 g. Larger single doses produced lightheadedness, anorexia and nausea. Further studies are planned to determine the optimum regimen for therapeutic trials.

(4) *Pharmacology.* WR 194965 appears to be moderately well absorbed in animals. Its half-life is about 10 days in monkeys. This prolonged effect has been confirmed by efficacy studies in owl monkeys; the trials showed that single oral doses are as effective as the same total dose divided into 7 equal daily doses.

Dihydrotriazines



Drug	Substitutions	Primary mouse CD_{50} (mg/kg)	Owl monkey CD_{50} (mg/kg)	Human curative dose (g/man)
WR 38839	3 = Cl, 4 = Cl, R = CH_2	127	35	1 8
WR 99210	2 = Cl, 4 = Cl, 5 = Cl, R = $O(CH_2)_3$	307	6 1	

(1) *General.* Only one dihydrotriazine has been tested for antimalarial activity in man. A related analogue was developed up to the stage of clinical testing but it was not thought to be sufficiently bioavailable for therapeutic effect.

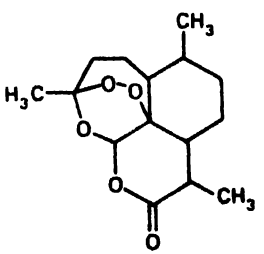
(2) *Spectrum of activity.* The dihydrotriazines are effective against blood schizonts of both falciparum and vivax malaria. There is some evidence that, when combined with a sulfonamide, they exert causal prophylactic activity against *P. falciparum.* WR 38839 is cross-resistant with pyrimethamine, whereas WR 99210 is not.

(3) *Toxicity.* The dihydrotriazines are only moderately well tolerated. The dose-limiting factors have been gastrointestinal symptoms with both WR 38839 and WR 99210. At the doses tested, no evidence of "antifol" toxicity has been observed.

(4) *Pharmacology. In vitro,* the Uganda strain was much more susceptible to WR 38839 than the Camp and Marks strains, yet the parasites of both the drug-resistant strains were markedly affected at a concentration of the drug of only 50 μg/l blood. Drug concentrations of this order were maintained for no more than 4 hours during the daily dosage.

(5) *Clinical results.* WR 38839 showed significant blood schizontocidal activity against a pyrimethamine-sensitive strain of *P. falciparum* in partially immune African children. It was also active against the Uganda 1 strain of *P. falciparum.* Infections of nonimmune volunteers with this strain were cured by the oral administration of 0.6 g of the drug daily for 3 days, but about double this dose failed to cure infections with the Malaya (Camp) or the Viet Nam (Marks) strain. Administration of the drug in combination with sulfadiazine cured half the patients with drug-resistant falciparum malaria.

Sesquiterpene Lactones



(1) *General.* Qing hao su is the antimalarial constituent extracted from the plant *Artemisia annua L* in 1972 at the Chinese Institute of Materia Medica.[3] It has been formulated and tested widely in China in both oral and parenteral forms.

(2) *Spectrum of activity.* Qing hao su is effective against asexual blood forms. There is no evidence of activity against tissue stages. Studies in both chloroquine-sensitive and chloroquine-resistant *P. berghei* in mice suggest some degree of cross-resistance with chloroquine. However, studies in patients with *P. falciparum* do not show any evidence of chloroquine cross-resistance.

(3) *Toxicity.* The drug appears to be remarkably well tolerated in man. Occasionally, slight pain has been recorded at the site of parenteral injections.

(4) *Pharmacology.* Qing hao su appears to be rapidly absorbed in animals. Using radiolabelled drug, peak concentrations are reached in 1 hour and the half-life of the radioactivity is about 4 hours.

(5) *Clinical results.* Results of clinical studies administering Qing hao su have been reported on over 2000 malaria cases, of which over 1500 were *P. vivax* and over 500 *P. falciparum* infections. In addition, 143 cases of chloroquine-resistant *P. falciparum* and 141 cases of cerebral malaria have been treated by Qing hao su. For these clinical trials, the drug was formulated in the form of tablets, oil suspension and water suspension and the total dosage administered per adult was: tablets 2.5 - 3.2 g; oil suspension 0.8–1.2 g and water suspension 1.2 g.

The conclusion made by several research groups was that Qing hao su is a schizontocide with very low toxicity. It is effective against *P. vivax* and both chloroquine-sensitive and resistant strains of *P. falciparum* respond well to the drug. The rate of recrudescence (up to 30 %) occurring 15–30 days after the initial treatment is reduced when the drug is administered intramuscularly in the form of oil suspension.

Comments

The development of antimalarial drugs is costly and time-consuming, but to combat malaria newer and better drugs will be required for the foreseeable future. Various new potential antimalarial drugs are under development, at least one of which, mefloquine, has been developed to the stage where medical practitioners using it to treat chloroquine-resistant falciparum malaria may reasonably expect a dramatic increase in their ability to cure it.

[3] Antimalaria studies of Qing hao su; Chinese Institute of Materia Medica, Academy of Traditional Chinese Medicine (unpublished document, March 1979).

CHAPTER 5

DRUG RESISTANCE IN MALARIA

Definition of Drug Resistance and Gradation of Response to Antimalarials

The factor limiting success in the use of antimalarial drugs, whether for prophylactic or for curative purposes, is the varying response of the species or strains of the parasite. Each drug in use has been selected because it has one or more specific actions against the malaria parasite when administered to the patient in the appropriate dosage. When this recognized action does not occur, it may be that not enough of the drug or its active metabolite has reached the parasite (in which event we are dealing with drug failure), or the drug has reached the parasite but the parasite has become adapted to the introduced chemical environment and, by surviving, has entered the state of drug resistance.

Definition of resistance

Drug resistance in malaria has been defined as the "ability of a parasite strain to survive and/or to multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the limits of tolerance of the subject." (WHO, 1965, 1973). This definition must now be qualified in the light of present knowledge of sulfonamide metabolism in certain individuals, with a statement to the effect that the form of the drug active against the parasite must gain access to the parasite or the infected red blood cell for the duration of time necessary for its normal action. This qualification depends on certain observations: (*a*) in some individuals sulfonamides or sulfones may be metabolized abnormally—for example becoming bound to protein—and released too slowly to maintain an effective antiparasitic level; (*b*) parasites resistant to chloroquine may not encounter lethal quantities of this drug because the membrane of the host red blood cell and the parasite membrane become selectively impermeable to drug owing to the abnormally high pH of the metabolic products of the parasite—lactate production by chloroquine-susceptible parasites reduces the pH of the red blood cell, thus inducing absorption of the drug. This is, however, only part of a complex process.

Although drug resistance embraces all species of malaria parasites and all acceptable dosages of blood or tissue schizontocides, gametocytocides and

sporontocides, in practice it is most commonly related to the effect of blood schizontocides on falciparum malaria. Within the latter category, furthermore, the unadorned term "drug-resistant malaria" is at the present time customarily understood to refer to the 4-aminoquinolines, particularly chloroquine.

Differing susceptibility of species, strains and stages of parasites

Species of human malaria parasites differ in their susceptibility to drugs in circumstances where resistance, as defined above, cannot be invoked. Thus, falciparum infections are more susceptible to sulfonamides than other species of human plasmodia. In mixed infections treated with chloroquine, trophozoites of *P. malariae* tend to linger in the blood after those of *P. falciparum* have been cleared (a factor being the slower developmental cycle of the former), and this may be a confusing element in the field application of *in vitro* drug susceptibility tests.

Strains of a particular species may differ in their response to a drug. For example, relapses of infection with the Chesson strain of *P. vivax* occur following treatment of some patients with 14 doses of primaquine, 15 mg (base) being given each day, whereas this regimen produces radical cure when other strains of the same species are involved. For 50 years or more it has been known that some strains of *P. falciparum* have a lessened sensitivity to quinine; however, the distinction between this so-called "natural" phenomenon and the type of resistance described in this chapter is sufficiently arbitrary for them to be presented together in the section relating to quinine.

The stages of a malaria parasite differ markedly in their susceptibility to different drugs. It is apparent that the effectiveness of a drug such as primaquine that interferes with stages as morphologically distinct as, for example, the *P. vivax* exoerythrocytic schizont and the *P. falciparum* gametocyte implies that those stages have at least partially similar metabolic processes. In terms of the definition given above, the "normal" failure of primaquine to interfere with asexual parasitaemia does not imply drug resistance. However, when a stage of parasite normally susceptible to a drug loses its susceptibility and resistance (as defined) develops, then all other stages of that parasite strain become resistant to the drug. Fortunately primaquine has not yet stimulated this kind of resistance, but resistance of the asexual stage of *P. falciparum* to proguanil or pyrimethamine has been accompanied by resistance to their sporontocidal action.

Gradation of response to drugs

Implicit in the definition of drug resistance is a gradation or spectrum of response by the asexual parasite, from mere survival at a subpatent level with subsequent recrudescence in the blood to active multiplication during the course of treatment. A system of grading the responses of asexual

P. falciparum to normally recommended doses of chloroquine has been proposed (Table 6 and Fig. 27) and has proved practical and useful. Although it may be applicable also to other blood schizontocides and other species, it may require modification in relation to the speed of action of the particular drug and the biological characteristics of the plasmodial species. A modifying effect may also be exerted by the immune status of the patient, with the result that a parasite response, even a partial one, to chloroquine will be more rapid in a semi-immune than in a nonimmune person, and a symptomatic response will be even more prompt.

Resistance to the Common Antimalarial Drugs

4-aminoquinolines: chloroquine

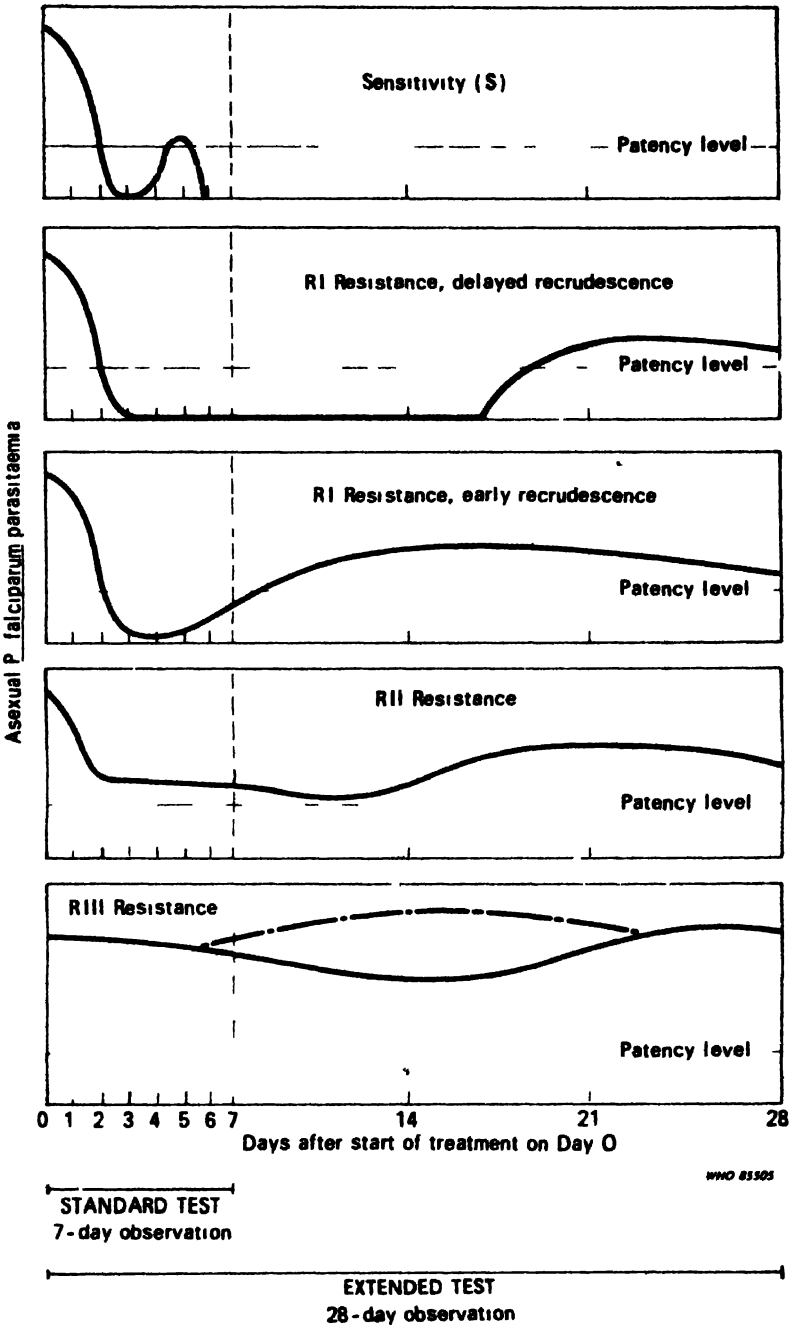
At present the only species of human malaria parasite that has developed resistance to chloroquine is *P. falciparum*. The degree of resistance (stated in terms of the grading system shown in Table 6 and Fig. 27) has often been RI in newly established foci, while responses graded as RIII may occur in strains tested from areas where resistance is widespread and of long duration. In this connexion it should be noted that strains showing RI response patterns to curative treatment with 1.5 g chloroquine (base) will often be held to a subpatent level in people receiving 0.3 g (300 mg) (base) prophylactically each week, but may emerge when prophylaxis ceases. Strains with an RII or RIII response to curative treatment break through such prophylactic treatment without even a lengthening of the normal prepatent period.

The geographical distribution of chloroquine-resistant falciparum malaria has increased greatly since it was first suspected in Thailand in 1957 and found in patients in Colombia in 1960. Since then, resistant strains have appeared in much of south-east Asia, where sensitive parasites have been replaced to such an extent that in Thailand, for example, surveys indicate that the majority of infections are chloroquine-resistant. The same has probably occurred in those parts of South America where malaria eradication programmes have not been successful. Although it is possible that these strains were previously present

TABLE 6 GRADING OF RESISTANCE OF ASEXUAL PARASITES (*P FALCIPARUM*) TO SCHIZONTOCIDAL DRUGS (4-AMINOQUINOLINES)

Response	Recommended symbol	Evidence
Sensitivity	S	Clearance of asexual parasitaemia within 7 days of initiation of treatment without subsequent recrudescence
Resistance	RI	Clearance of asexual parasitaemia as in sensitivity followed by recrudescence
	RII	Marked reduction of asexual parasitaemia but no clearance
	RIII	No marked reduction of asexual parasitaemia

Fig 27 Response to field test for sensitivity of falciparum malaria to chloroquine[1]



From WHO Technical Report Series, No 529, 1973 (amended)

and undiagnosed in some of the areas, the evidence indicates that there has been a considerable geographical extension from one or more of the originally identified foci, the migration of semi-immune carriers to receptive areas playing an important part. It is apparent that the strain infiltrates into, and becomes established among, the drug-susceptible falciparum infections endemic in the rural population a year or more before it is detected by a conspicuous failure in the chloroquine treatment of a case at a hospital or clinic. In the affected countries, resistant and sensitive strains are so mixed that some cases of falciparum malaria may still be cured with the usual 3-day course of chloroquine.

The distribution of chloroquine resistance in 1980 in Asia was as follows (Fig. 28):

(1) Much resistance, often of a high grade, with local transmission in Burma, Malaysia (Peninsular and Sabah), the Democratic Republic of Kampuchea, Lao People's Democratic Republic, Socialist Republic of Viet Nam, Thailand, Papua New Guinea, and the Philippines.

(2) Scattered foci, usually of moderate grade, with local transmission in Indonesia (Kalimantan, Irian Jaya, Sumatra), southern China, Bangladesh, India (north-eastern states, Orissa), Vanuatu, and the Solomon Islands.

(3) Occasional cases in migrants, contracted elsewhere and not transmitted locally, in Nepal and Malaysia (Sarawak).

In South America, in those parts of Brazil, Colombia, French Guiana, Guyana and Suriname where transmission continues, *P. falciparum* is often resistant to chloroquine. Small foci occur in Bolivia, Ecuador and Venezuela. Southern Panama has become involved and, depending on the efficiency of the national malaria eradication programmes, other countries of Middle America are threatened (Fig. 29). The increase in relative prevalence and in the degree of resistance of *P. falciparum* to chloroquine is greater in south-east Asia than in South America.

In Africa, strains of *P. falciparum* in Ethiopia, the Sudan and possibly Nigeria are somewhat less sensitive to chloroquine than are other African strains, and it has been suggested that this represents innate strain characteristics rather than strain selection under drug pressure, although they still respond to the normal therapeutic regimen. Isolated cases apparently resistant to chloroquine at the R1 level have been observed in apparently non-immune travellers who had contracted falciparum infections in Kenya and the United Republic of Tanzania (Center for Disease Control, 1978). Investigations in Kenya indicate a significant lowering of chloroquine susceptibility levels between 1972 and 1979. Occasional resistance of *P. falciparum* to chloroquine has also been reported from Madagascar and Zambia, but confirmation is pending. Recently, however, a strain of *P. falciparum* isolated from a visitor to the United Republic of Tanzania showed a high degree of resistance to chloroquine (Campbell et al., 1979).

Fig. 28 Distribution of confirmed foci and areas of resistance of *P falciparum* to chloroquine in Asia

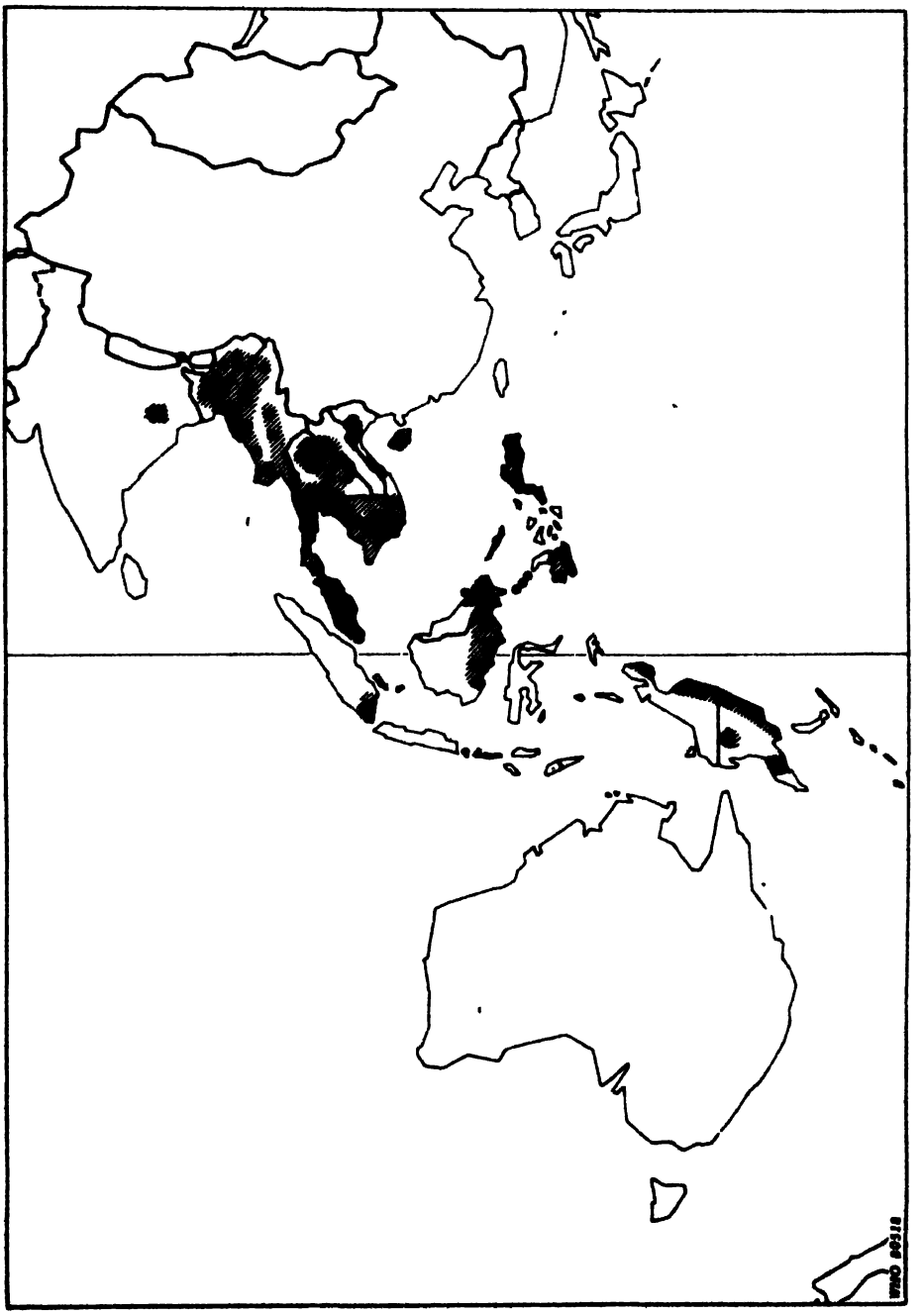
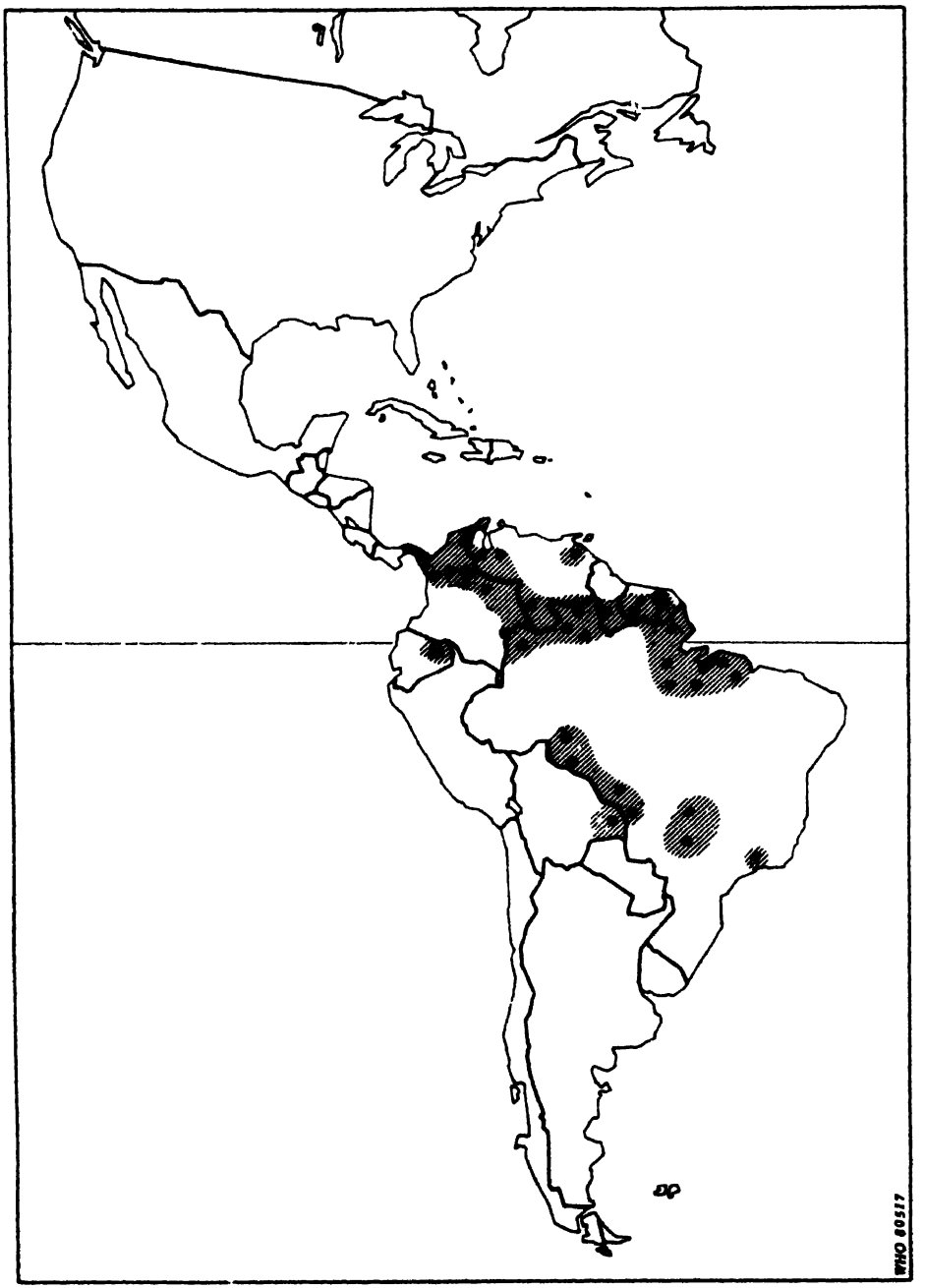


Fig 29 Distribution of confirmed foci and areas of resistance of *P falciparum* to chloroquine in South and Middle America



A major global effort to monitor the sensitivity of *P falciparum* to antimalarial drugs has recently been mounted under the auspices of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases

4-aminoquinolines amodiaquine

Strains of *P falciparum* resistant to chloroquine are not necessarily also resistant to the closely related compound amodiaquine *P vivax* and *P malariae* have not been found to be resistant to these drugs *In vivo* and *in vitro* testing of a number of strains of *P falciparum* from south-east Asia and South America indicates that equivalent doses of amodiaquine are usually more effective than chloroquine for curative purposes, with the possible exception of some Philippine strains found primarily to be resistant to amodiaquine There is no evidence that amodiaquine is effective for the prophylaxis of chloroquine-resistant *P falciparum* Irrespective of the Philippine situation, the suggestion that amodiaquine might be used to combat *P falciparum* in areas of chloroquine resistance cannot be supported

The geographical distribution of amodiaquine-resistant falciparum malaria resembles that of chloroquine-resistant falciparum malaria

Quinine

Many years before chloroquine-resistant strains of *P falciparum* were found, which in some instances have a reduced sensitivity to quinine, variations were encountered in the response to quinine These variations, presumed to be natural, were reported from several parts of the world An Italian strain was shown to be less sensitive than an Indian one observed under the same conditions, and a strain from Panama was reported to have shown a mild relative resistance In general, from the Indian subcontinent westward through Africa very sensitive parasites occurred, but in south-east Asia and the Americas they were relatively resistant In many instances, however, it was uncertain whether the patient's response (vomiting, malabsorption, idiosyncrasy) or the parasite's response should be held accountable for the variations in dosage required for cure In the many well-known examples of alleged "quinine-resistant" malaria reported from Mesopotamia after the First World War, the patient's response and in some cases the accuracy of the identification of the parasite species have been questioned. Although vivax malaria responds generally less quickly to quinine than do infections caused by sensitive strains of *P falciparum*, resistance to this drug has not been seen in *P. vivax* or *P malariae*

In the response of *P. falciparum* to quinine, it is sometimes difficult to distinguish between strain variations and true resistance. More certainly in the latter category were parasites responsible for infections occurring in Brazil in 1908–1910, where dosage regimens of quinine as high as 25 5 g base

given over 21 days were not producing a cure. In some areas of relative quinine resistance (e.g. the island of New Guinea) strains of *P. falciparum* resistant to mepacrine and to the 4-aminoquinolines have been reported.[1] In contrast, in the originally quinine-hypersensitive areas such as sub-Saharan Africa, chloroquine resistance, although it may have appeared in isolated individual cases, has not become established despite the distribution, often in an irregular and haphazard manner, of vast amounts of that drug.

Quinine resistance is associated with chloroquine resistance, several strains of *P. falciparum* highly resistant to chloroquine being cross-resistant to quinine. Responses in the RI category to the 14-day course of quinine (2 g daily) occur with several strains, and cases of RIII resistance at this high dosage level have been encountered very rarely, notably in a strain from Viet Nam. During studies of one strain from Peninsular Malaysia, an increase in its degree of resistance to quinine was reported. It is interesting to note that long courses of quinine given by slow intravenous infusion to patients infected with quinine-resistant *P. falciparum* have attained a much higher cure rate than the same courses given by mouth.

Quinine-resistant falciparum malaria probably occurs within or close to those areas where *P. falciparum* shows a high degree of resistance to chloroquine

Primaquine

It is important to observe that primaquine continues to exert its expected gametocytocidal and sporontocidal effect on all strains of *P. falciparum* so far studied, including those most highly resistant to the 4-aminoquinolines and quinine.

However, strains of *P. vivax* vary in their response to the tissue schizontocidal action of primaquine. Adults infected with the Chesson-type strain, which was first seen in the island of New Guinea in 1944 and by 1978 was present also in the Solomon Islands, Indonesia, and Thailand, should receive a total dose of primaquine of 6.0 mg (base) per kg body weight to prevent relapse, this being twice the quantity needed to eliminate other strains.

Proguanil, pyrimethamine and related compounds

The dihydrofolate reductase inhibitors such as proguanil and pyrimethamine used to hold an important place in the prophylaxis of malaria, particularly where *P. vivax* and *P. malariae* predominate. Resistance of *P. falciparum* to one or both is present in certain localities of all the endemic regions, including Africa. Failure of these compounds to maintain their initial

[1] The present distribution of chloroquine-resistant strains extends eastwards in the north and the south parts of Papua New Guinea from the border of Irian Jaya (Indonesia).

successful effects was observed within 2 years of their introduction and should now be anticipated in any areas where the drugs are or have been used on a large scale. Cross-resistance between proguanil and pyrimethamine is often, but not always, present. For the prophylaxis of falciparum malaria, an increase of the daily dose of proguanil to 200 mg has proved effective in areas where the infections break through 100 mg of proguanil given daily or 25 mg of pyrimethamine given weekly. It appears that all stages of the parasite normally affected by these drugs become resistant, with the result that the therapeutic, prophylactic and sporontocidal effects are lost simultaneously.[2]

Drugs related to proguanil, for example chlorproguanil and the long-acting repository compound cycloguanil, are unlikely to be effective against proguanil-resistant strains of *P. falciparum*, although trimethoprim may be an exception since cross-resistance or resistance to this drug has not yet been demonstrated. The apparent ease with which *P. falciparum* resistance to this group of drugs can develop, appearing after as little as a single exposure of the parasites to one of the compounds, has been shown experimentally in both nonimmune and semi-immune subjects in the field, and is a contraindication to their large-scale distribution alone or as medicated salt.

On the other hand, resistance by *P. vivax* or *P. malariae* to proguanil and pyrimethamine is less frequent. Resistant strains of these two species have been produced experimentally. In the field in Peninsular Malaysia *P. vivax* resistant to proguanil was observed in 1950, and in the Province of Taiwan (China) and Java (Indonesia) *P. malariae* appeared to respond in similar fashion. *P. vivax* was found insusceptible to pyrimethamine in a focus in Venezuela (possibly imported from Colombia), in Kenya and in Pakistan, while recently a strain from Viet Nam transferred to the owl monkey proved to be highly resistant and may occur in humans in its original area. A single case of *P. malariae* infection in Kenya was also insusceptible to pyrimethamine.

The geographical distribution of proguanil-resistant and pyrimethamine-resistant *P. falciparum* is widespread, foci occurring in all the endemic regions and particularly in places where mass drug distribution has occurred. Unlike chloroquine resistance, which appears to have originated in a limited number of places in south-east Asia and South America and spread outwards, only recently emerging in Africa despite the large-scale use of chloroquine in several areas, resistance by *P. falciparum* to proguanil and pyrimethamine has appeared soon after the start of large or small distribution schemes in Africa and elsewhere. It seems to become dominant over susceptible strains and (after the drug pressure has been removed) appears to subside gradually into the resurgent mass of susceptible strains.

[2] However, an early study of the response of one strain of *P. falciparum* from Malaysia showed that trophozoite resistance to proguanil did not affect the sensitivity of pre-erythrocytic stages of this particular strain.

Sulfonamides and sulfones

Resistance of the *P. gallinaceum* of birds and the *P. berghei* of rodents to sulfonamides and sulfones is well known experimentally. Although there are several published reports referring to such resistance on the part of *P. falciparum*, it is considered that the present position regarding this species will remain uncertain until confirmation in isolated and subinoculated strains is obtained. Indeed, in some instances it appears that failure to cure infections (even those most highly resistant to the 4-aminoquinolines, quinine, proguanil and pyrimethamine) may be attributable to metabolic factors affecting the drug in certain people, and not to the presence of resistant characteristics in the parasite.

Nevertheless, the evidence of true resistance to the sulfonamides and sulfones appearing in *P. falciparum* should not be overlooked, as the action of these drugs in blocking para-aminobenzoic acid and thus interfering with parasite metabolism has been circumvented by several other species of plasmodium. Field observations indicate that strains of *P. falciparum* resistant to the combination of sulfadoxine with pyrimethamine have appeared in some parts of south-east Asia and of South America. Other reports from these areas stress that the response of *P. vivax* malaria to the combination of sulfadoxine with pyrimethamine has been much less satisfactory than in *P. falciparum* infections. Thus chloroquine followed by primaquine is still the best treatment of vivax malaria.

Origin and Mechanism of Drug Resistance

Resistance by *P. falciparum* to chloroquine, as well as by all species to proguanil and pyrimethamine, is attributable to selection under drug pressure of resistant mutants which survive by utilizing alternative metabolic pathways to those blocked by the particular drug. In respect of chloroquine, resistance is characterized by a decrease in high-affinity binding sites for the drug. Once selected, and provided that they escape the destructive action of host immunity, the resistant parasites may be transmitted by local mosquitos to other people in the immediate area, or may be carried by a migrant host to other places where mosquitos may or may not be present to establish transmission. There appears to be no difference in the susceptibility of anophelines to drug-resistant and drug-sensitive strains of *P. falciparum*. Thus, for example, *Anopheles stephensi* from western Asia and *A. arabiensis* (formerly *A. gambiae* B) from Kano, Nigeria, collected in areas far distant from known foci of chloroquine resistance, were found experimentally to be highly efficient vectors of multidrug-resistant south-east Asian strains.

Many variables related to the host, the vector and the treatment affect the selection, survival and local or distant propagation of drug-resistant malaria parasites. Selection for drug resistance is more likely if large numbers of infected people are treated, if treatment continues for a long time, and if many

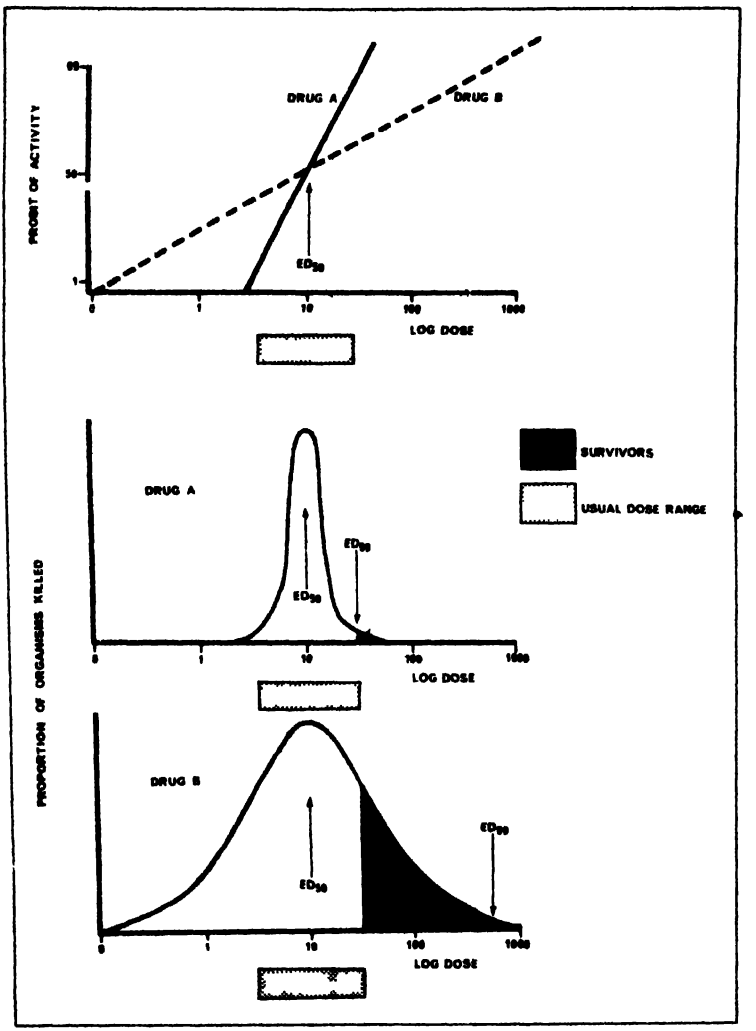
parasites are exposed to the drug in each patient. Propagation of a resistant strain depends on a coincidence of favourable epidemiological circumstances, widespread use of the drug to which resistance has been acquired being an important factor in restraining competition from local drug-susceptible strains of the parasite. In the course of mosquito transmission, genetic recombination of the sexual stages of parasites may occur if the mosquito has simultaneously ingested two or more strains of gametocytes circulating in one person or has divided its feed between people carrying different strains: hybridization between differing strains may result in modification of the degree of drug sensitivity. In the absence of hybridization, however, resistance by a strain of *P. falciparum* to chloroquine survives numerous mosquito passages undiminished. This stability of chloroquine-resistant *P. falciparum* in both the mosquito and the human host, in whom it may outgrow drug-sensitive strains in the absence of drug pressure, contrasts with the tendency of pyrimethamine-resistant strains to recede when drug pressure is removed, their place being taken by pyrimethamine-sensitive strains.

Resistance to proguanil and pyrimethamine developed soon after these compounds began to be used, but chloroquine resistance did not appear for many years and remains largely absent from the most highly malarious region, Africa. The reasons for this contrast appear to be at least twofold. First, inhibition by proguanil and pyrimethamine of dihydrofolate reductase is a metabolic block apparently more readily circumvented by mutant parasites than are the complex actions of the 4-aminoquinolines. Second, the dose-activity responses of these groups of compounds differ markedly (Fig. 30). In the top graph of this figure, the dose-activity relationship of chloroquine (Drug A) is represented by a steep line, while that of proguanil (Drug B) is flatter. Peters (1969) comments as follows about chloroquine:

"By making a small increase in the dosage the activity can be changed from, say 1 % to 99 % and it is easy to envisage that most of the parasites may be killed by a dose that is still well within the tolerated dose. The chances of a rare resistant organism surviving the maximum tolerated dose are thus very small. On the contrary in the case, for example, of proguanil, a very considerable increase must be made in the dose to increase the efficiency of the drug (which is in any case only plasmodistatic and not plasmodicidal) from 1 % to 99 % and it is manifestly impossible to raise it enough to produce a 100 % effect. Thus it is relatively easy for parasites that can withstand, say, more than the ED99 to survive. Assuming in each case that the average dose administered represents about the ED50 this clearly offers a far higher chance of survival to mutants that are resistant to proguanil than to those that are resistant to chloroquine when proguanil or chloroquine respectively are the drugs in question. Seen as a function of time, it will obviously take longer for any pre-existing chloroquine-resistant mutants to emerge than those resistant to proguanil."

In this connexion, the passage of small amounts of pyrimethamine in breast milk from treated mothers to infants encountering their first infections, which

Fig 30 Dose activity response curves of 2 groups of antimalarial compounds that of chloroquine (drug A) and that of proguanil (drug B)[1]



Redrawn from Peters W (1969) *Transactions of the Royal Society of Tropical Medicine and Hygiene* **63** 25

have high parasite densities, exposes a wide spectrum of mutant parasites to subtherapeutic quantities of drug, an ideal situation for the selection of resistant strains High levels of immunity in potential hosts discriminate against the few parasites representing the first wave of resistance to chloroquine, of the much larger initial number resisting proguanil or

pyrimethamine, some parasites survive the incomplete toll of acquired immunity. The higher levels of host immunity in Africans than in the inhabitants of south-east Asia and South America may account to some extent for the slowness of transmission of chloroquine-resistant *P. falciparum* that may already have been introduced into Africa. The suggestion that strains of *P. falciparum* indigenous to Africa and western Asia (areas historically containing parasites hypersensitive to quinine) are incapable of producing mutants resistant to chloroquine has recently been refuted experimentally by the development of this resistance *in vitro* in a strain from the Gambia.

Recognition of Resistance

The failure of a course of treatment to exert its expected effect on a malaria infection may be reported from a hospital or clinic, suspected following an abnormal increase in cases during presumptive treatment, or found through deliberate drug testing. It may also be observed in cases of malaria imported from a part of the world where the phenomenon of drug resistance is widespread. In places from which reports of suspected resistance have been received, and also from areas where drug responses appear normal, it is essential to obtain baseline information on the degree of susceptibility of the parasite to antimalarial drugs.

Several procedures are available for use in the field and clinic. The selection of the appropriate method depends on the level of immunity of the subjects to be examined, their clinical condition, the period of time within which they can be followed up, and their chances of becoming reinfected during the observation period. The procedures designed for the detection of chloroquine resistance in *P. falciparum* but adaptable to other schizontocides are the following (see Fig. 27 and Annex 5).

(1) the standard field test, giving 25 mg/kg chloroquine (base) over 3 days (10 mg/kg on each of the first two days, 5 mg/kg on the third day) with a 7-day observation period ("7-day test");

(2) the same test, with the observation period extended over a total of 28 days ("extended test"). This is used in preference to (1) only if the patient is not exposed to new infection for 28 days;

(3) the single-dose test ("alternative test"), giving 10 mg/kg chloroquine (base). This test may be applied in the following circumstances:

(i) where for any reason treatment for 3 days cannot be given,

(ii) in areas of high endemicity where, owing to the high level of immunity in the population, this dose has been accepted as the standard form of treatment, and

(iii) as a preliminary screening procedure prior to applying the standard 3-day treatment.

Although there is some possibility of vomiting after the first dose, particularly if it is given on an empty stomach, chloroquine is administered by mouth in preference to injection because of the safety, ease of administration, and uniformity that this route provides. The test is evaluated by the examination of thick blood films, since a fall in pyrexia is not a reliable criterion of the activity of the drug on the parasite.

Since transmission cannot always be excluded under field conditions, recrudescences cannot always be distinguished from reinfections. Resistance at the RII and RIII level is therefore based on the response of asexual parasitaemia during the first week of treatment. Only if new infections can be excluded will further observations during an additional 3 weeks (the extended test) yield more conclusive evidence on the recrudescence of parasitaemia, thus permitting the observer to distinguish between sensitivity (S) and the RI level of resistance.

For obvious reasons severely ill patients should be excluded from the test and given alternative treatment such as quinine. Persons with mixed infections, particularly *P. malariae* which, though drug-sensitive, may persist for 7 days, should also be excluded. It is desirable to include persons with high parasite counts, in practice, this will mean young children in highly endemic regions. Whenever possible the urinary excretion of chloroquine should be determined (see Annex 4).

Interpretation of the field tests

(1) If no asexual parasites are found by day 6 and none are present on day 7 the infection may be either sensitive (S) or resistant at the RI level. When fresh infection can be excluded for 28 days, the extended test being used, failure of the parasites to reappear by day 28 indicates that they are sensitive (S).

(2) If asexual parasites disappear for at least 2 consecutive days but return and are present on day 7, they are resistant at the RI level (7-day test). When fresh infection can be excluded for 28 days, the extended test being used, any asexual parasite recrudescence within 28 days indicates an RI response.

(3) If asexual parasitaemia does not clear but is reduced to 25 % or less of the original pretest level during the first 48 hours of treatment, the parasites are resistant at the RII level.

(4) If reduction of the asexual parasitaemia is less than 75 % during the first 48 hours, if it remains at the same level, or if it continues to rise, the parasites are resistant to the standard dose of the drug at the RIII level.

Trials in hospitals and reference centres

Resistance to schizontocidal drugs in falciparum malaria may be assessed not only from field trials but also from clinical tests of the response of patients treated for acute infections in hospitals and specialized reference centres, where the opportunity exists for extended observation under conditions

excluding the chance of reinfection It is probable that the high levels of parasitaemia usual in acute infections present a better test of sensitivity or resistance than do the lower levels common in symptomless carriers Adaptation of *P falciparum* to the owl monkey has been achieved and offers a valuable alternative means of studying the sensitivity of human malaria parasites to drugs *in vivo*

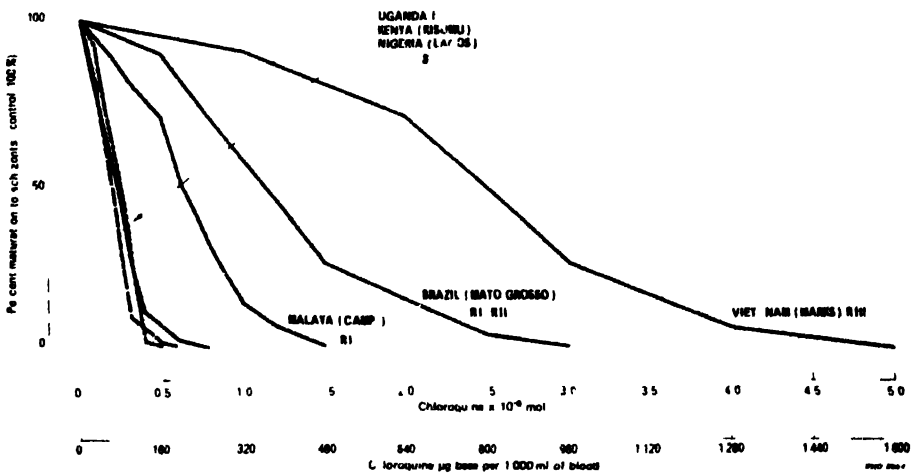
In order to relate the important observations in the field to the detailed characterization of strains in human malaria research centres, close coordination between these two sources of data is essential

In vitro Test for Blood Schizontocidal Action against *P. falciparum*

The problems inherent in conducting field and hospital tests have shown the necessity of developing an *in vitro* test that can be used to study the response of *P falciparum* to various schizontocides Testing samples of infected blood *in vitro* minimizes the variations in the apparent drug response due to immunity as well as the operational difficulties of dealing with patients who cannot readily be followed up

The maturation of parasites *in vitro* is inhibited by 4-aminoquinolines such as chloroquine and amodiaquine and dihydrofolate reductase inhibitors such as pyrimethamine and cycloguanil The extent of this inhibition can be assessed by comparing the degree of maturation in control samples of blood with that observed in samples containing the drug The percentage of ring

Fig 31 Chloroquine sensitivity of *P falciparum in vitro* and *in vivo*[1]



From WHO Technical Report Series No 529 1973

forms that mature to normal-looking schizonts containing more than 2 nuclei provides a useful endpoint for the quantitative assessment of maturation (see Annex 6).

This test has been used extensively in the field in recent years, and has proved valuable for determining the response patterns of *P. falciparum* to chloroquine and other drugs. It has recently been adapted to a micro-technique (see Annex 6), the collection of samples of capillary instead of venous blood being much more acceptable to the populations of many of the affected areas.

Preliminary results have shown a good correlation with the *in vivo* test, particularly as concerns sensitivity and the RI and RIII types of resistance (Fig. 31). However, more field studies with the *in vitro* and *in vivo* tests performed in parallel are required to ascertain to what degree the *in vitro* test is a dependable indicator of the level of drug response in a given area.

CHAPTER 6

THE CLINICAL USE OF ANTIMALARIAL DRUGS

Although cinchona powder, the first specific remedy against "ague", was introduced into medicine in the early seventeenth century, its use was often haphazard and unsatisfactory. The reasons for this, as stressed by Russell (1955), were multiple. Confusion between various plants and cinchona, fraudulent adulteration of the genuine "Jesuit's bark", an insufficient dosage and the lack of an accurate diagnosis of malaria infections were some of the reasons.

But even in the middle of the nineteenth century, when quinine became widely available, the results of treatment were not always satisfactory, for other reasons. Some of them were the different responses of the successive developmental stages of human plasmodia, the presence of four different species, the existence of various geographical strains within one species, the vagaries of the therapeutic outcome depending on the degree of immunity of individuals and large groups, and the lack of understanding of the phenomena of relapse.

The twentieth century provided a series of synthetic antimalarials of outstanding value, explained the mystery of the exoerythrocytic phase of development of human plasmodia and unravelled some of the subtle and complex aspects of the immune response. But the therapeutic outlook for malaria chemotherapy was clouded over once again by the appearance and spread of various degrees of resistance in human plasmodia to most of the synthetic compounds. Moreover, as more active antimalarial drugs were developed and their use spread, various adverse effects of treatment or of long-term prophylaxis were reported, at times with little justification.

However, it seems at present that many therapeutic errors in the treatment of malaria are attributable to delayed or missed diagnosis of the disease, especially in many developed countries, whose medical professions are not familiar with the protean manifestations of this or other tropical infections. In view of this a brief description of the symptoms and the variations in the clinical course of malaria may not be out of place.

Clinical Course of Malaria

After an incubation period ranging between 7 and 37 days for most cases of naturally transmitted malaria (with some strains of *P. vivax* the

incubation period may be delayed for several months), the first premonitory signs appear more or less suddenly, consisting of headache, malaise, anorexia, nausea, muscular pains, fatigue and dizziness However, it should be emphasized that in many instances persons who have been taking prophylactic drugs during their stay in a malarious area and for some time after their return to a temperate climate may show clinical symptoms of the infection within a year This applies particularly to infection with *P. vivax*, *P ovale* and *P malariae*, it is unusual in such conditions to see infections with *P falciparum* later than 3 months after return to a non-malarious country

A typical paroxysm starts with a feeling of cold accompanied by shivering (rigor), pallor and cyanosis, in children it may present as a convulsive seizure Other symptoms including dry cough, abdominal pain and vomiting may mislead the physician into diagnosing influenza or a gastrointestinal condition In malaria caused by the transfusion of blood from an infected donor various surgical complications may be suspected The fever may persist for several days before showing periodicity A typical paroxysm consists of a sequence of a cold stage, a hot stage and a sweating stage, which occurs every 48 hours with vivax and ovale malaria, every 72 hours with quartan malaria

Such classic paroxysms are far from common On the one hand, in persons who have had a previous malaria infection the reinfection may present modified and often relatively mild symptoms On the other hand, in nonimmune persons variations in the clinical course are frequent and in falciparum infection can be misleading and dangerous and end fatally Irregular fever with the usual indistinct prodromal symptoms is not seldom the only clinical picture of falciparum malaria Because of the rapid multiplication of the plasmodia of this species and their tendency to invade the internal organs, severe complications may appear with dramatic suddenness Drowsiness, coma, delirium, bloody diarrhoea, severe haemolytic anaemia, pulmonary oedema, hyperpyrexia, shock syndrome and renal failure indicate the involvement of various organs Rapid diagnosis and immediate adequate treatment are necessary in every case of falciparum malaria, especially in nonimmune persons

In children malaria infection may simulate many diseases as well as obscure surgical conditions

The details of the clinical and differential diagnosis of malaria will be found in standard textbooks It should again be stressed that early and accurate diagnosis of malaria in anyone with a fever of obscure origin is of paramount importance at present, when mass travel to endemic tropical areas has greatly increased the chances of infection, even if it consists only of a brief stay at an airport Exact information on the patient's travels and on his medical history should be obtained from all patients as a routine Information on the countries visited may also indicate the possibility of drug resistance in some infections

Although the finding of plasmodia in the blood establishes the diagnosis of the disease, a single negative report does not exclude the possibility of malaria.

Several thick blood films should be taken in suspected cases and examined by a competent microscopist. Correct identification of the species of malaria parasite gives much guidance for the treatment of the patient.

If rapid examination of blood films is not possible and the diagnosis of malaria has not been discarded, adequate treatment should never be delayed. The blood sample should be taken but treatment should be begun without waiting for the result of the examination.

In the case of confirmed falciparum malaria the disappearance of parasites after treatment should be checked by serial blood examinations (with assessment of the parasite density, if possible by parasite counts) made frequently, if possible daily, to monitor the results of treatment Once-weekly blood examinations should be carried out for 4 weeks following treatment, in order to detect any possible recrudescence of the infection Reappearance of parasitaemia in a patient in a non-malarious area may indicate either an insufficient drug dosage or resistance of the plasmodial strain to the specific chemotherapeutic compound

Many deaths have occurred and the disease has often been unduly prolonged through delay in the diagnosis and neglecting to provide rapid treatment. If it is recalled that a single erythrocytic schizont of *P falciparum* has an average of between 8 and 24 merozoites and that the multiplication of parasites is repeated at every developmental erythrocytic cycle of 48 hours, it is understandable that even a scanty initial infection can soon overwhelm the patient The proportion of parasitized red blood cells may rapidly reach 5 %, 10 % and even 20 % as seen in the blood film, though this does not provide full evidence of the concentration of parasites in the internal organs It appears that when the peripheral parasite count reaches 10 % of the erythrocytes the fatality rate of nonimmune patients is over 50 %, in spite of treatment, because of the involvement of the central nervous system

Treatment of Acute Malaria[1]

The main aim in treating acute malaria is to eliminate the erythrocytic forms of the plasmodia from the circulating blood and internal organs, where they cause the morbid symptoms However, a second and not less important task is to sustain the physiological condition of the patient by counteracting the effects of the disease, through various means Clinical vigilance and an appraisal of the individual condition of each patient are of paramount importance. The value of proper nursing cannot be overemphasized. The most useful therapeutic drugs are highly active schizontocides, namely quinine, the 4-aminoquinolines and, if there is drug resistance to the latter group, some

[1] When the treatment of malaria is carried out mainly by auxiliary personnel, dosage schedules must be established for the main drugs The guidance given in Fig 32 may be useful

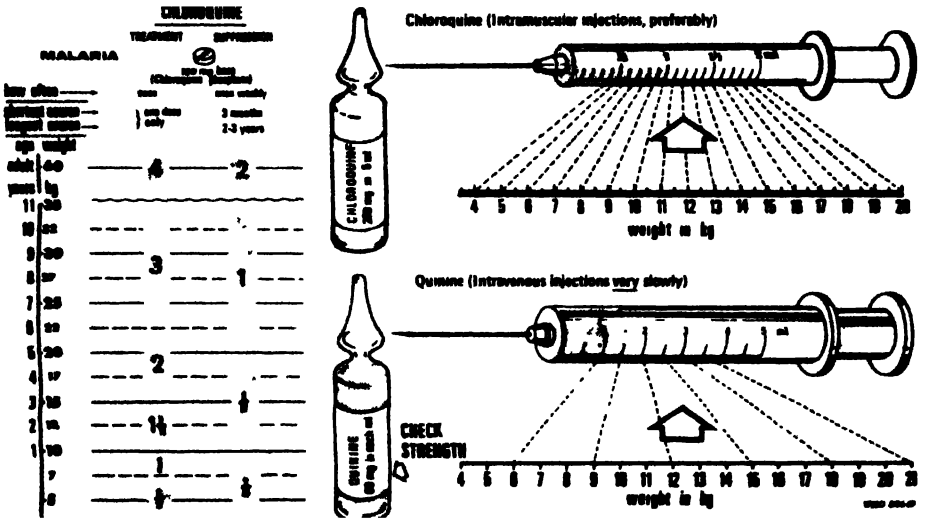
newer compounds. The initial treatment of acute malaria is the same irrespective of the species of parasite, except for some special circumstances such as severe infection with falciparum malaria in children or nonimmune subjects. It is the follow-up therapy of relapsing malaria that is different.

Strains of malaria parasites from different geographical areas may differ in their susceptibility to drugs, without exhibiting the phenomenon of true resistance. Consequently the therapeutic regimen must not be too rigid. Apart from possible variation in the drug dosage according to the weight of the patient, in clinical practice the judgement of the attending practitioner should be guided by the clinical symptoms and by the results of the blood examination for parasites and the haematological data. When the symptoms of acute malaria increase in severity, specific and general treatment should be instituted as indicated in the following section.

The selective value of certain antimalarial drugs for the treatment of acute infection with any of the 4 species of human plasmodia should be emphasized, as there seems to be some confusion in this respect.

Although somewhat displaced by modern synthetic antimalarials, quinine remains a potent and reliable drug. In the form of dihydrochloride or sulfate it may be given in solution, in capsules or in tablets. Some sugar-coated tablets may not be fully absorbed when the coating has hardened after long storage. Children tolerate quinine well and the proportional dosage should be at the upper limit of the range. However, for quinine to exert its full curative effect at

Fig 32 Injections of chloroquine and quinine[1]



By permission of the Oxford University Press from King, M. et al. (1978) *Primary child care*, Oxford, Oxford University Press, pp. 312, 315.

least a week or 10 days of treatment are needed and for this reason preference is usually given to the 4-aminoquinolines Chloroquine is generally the most effective therapeutic compound Its value and the rarity of adverse effects have been confirmed over 30 years of use worldwide This drug is available in the form of diphosphate or sulfate of the base under a large number of trade names, in the form of tablets with a base content ranging between 100 and 300 mg, or as a 4 °$_0$–5 °$_0$ solution for parenteral administration Amodiaquine and amopyroquine resemble chloroquine in their antiplasmodial action and there is some evidence that both drugs have a slightly greater activity against strains of *P falciparum* that show decreased susceptibility to chloroquine.

Primaquine, pyrimethamine and proguanil (or their derivatives) are not sufficiently effective as schizontocides for the treatment of acute malaria. On the other hand, various combinations of quinine with chloroquine, pyrimethamine, sulfones or sulfonamides have been used on patients whose response to chloroquine alone has been slow More information on the treatment of chloroquine-resistant falciparum malaria will be found on pp 131 - 132

The oral treatment generally advocated for moderately severe malaria in adults of average weight is shown in Table 7

In non-malarious areas, freedom from recrudescences of falciparum infection can be assured by follow-up treatment with 300 mg of chloroquine or amodiaquine, taken once a week for 1 month

In the above table mepacrine has been excluded, since this obsolete drug may cause various adverse effects However, if no other compounds are available and stocks of mepacrine can be located, the dosage of mepacrine is

TABLE 7 TREATMENT OF A SIMPLE ATTACK OF MALARIA IN AN ADULT OF AVERAGE 60 kg) BODY WEIGHT

	Chloroquine (base)	Amodiaquine (base)	Quinine
Day 1	600 mg	600 mg	
followed six hours later by	300 mg	200 mg	1800 mg (1950 mg) in divided doses
Day 2	300 mg	400 mg	1800 mg (1950 mg) in divided doses
Day 3	300 mg	400 mg	1800 mg (1950 mg)
Day 4	300 mg (if necessary)	400 mg (if necessary)	in divided doses
Days 5 to 7	300 mg (if necessary)	400 mg (if necessary)	1800 mg (1950 mg) in divided doses
Total dose	1500 mg of chloroquine base (more if indicated)	1600 mg of amodiaquine base (more if indicated)	12600 mg to 13830 mg over 7–10 days

[1] The dosage of amodiaquine shown in this column is based on the original content of 200 mg of amodiaquine base in the first proprietary formulations of this drug It appears that a new type of tablet containing 150 mg of amodiaquine base is now available In that case the regimen of amodiaquine treatment may be the same as that of chloroquine

[2] Most of the newer formulations of quinine salts are 300 mg per tablet The total dosage of quinine covers the range of treatment with 6 tablets daily for 7 days The dosage shown in brackets corresponds to the content of quinine in tablets still formulated at 10 grains or 650 mg per tablet

200 mg every 6 hours on the first day, followed by 300 mg daily for the next 6 days to a total dosage of 2 600 mg.

The table shows also the importance of the loading dose on the first day of treatment to achieve as soon as possible a high concentration of the drug in the plasma. This is less important with quinine, which has a more rapid bioavailability but is also rapidly excreted.

Most of these drugs, which are usually in the form of tablets, are intensely bitter, and some patients, especially children, may experience difficulty in swallowing them. They may be helped to do so by a generous drink of milk or fruit juice; as an alternative, the crushed tablets may be mixed with honey or jam. Care must be taken to ensure that the patient swallows the tablets and does not vomit later.

Antimalarial drugs can be administered per rectum with a satisfactory effect. This method is fairly common in several countries, though generally reserved for paediatric practice. For this type of treatment the dosage of 4-aminoquinolines in suppository form is double that for oral medication.

Treatment of Recrudescent or Relapsing Infection

Radical treatment aims at complete elimination of the malaria infection so that recrudescences or relapses cannot occur after treatment is completed. In falciparum infections an appropriate course of treatment with an effective blood schizontocide should result in the complete and permanent disappearance of the asexual forms of the plasmodia because this species has no latent exoerythrocytic stage.

The sexual forms of *P. falciparum* (male and female gametocytes) are not eliminated from the blood by the usual schizontocidal compounds.

In infections with *P. vivax*, *P. ovale* and *P. malariae* schizontocidal treatment will normally eliminate the trophozoites and gametocytes, but delayed relapses of vivax and ovale infections, owing to the presence of latent exoerythrocytic forms in the liver, will occur in the majority of patients.

The meaning of the two terms "recrudescence" and "relapse" is related to the source of the renewed plasmodial activity, though in practice sharp clinical differentiation between the two may not be easy. The reappearance of symptoms and of plasmodia in the blood may be due to different causes. First, treatment might have failed to eliminate all the malaria parasites for the reason that the dosage of the appropriate drug was inadequate, because of the error of the person responsible for the treatment, because of the non-compliance of the patient with the prescribed regimen, or because part of the ingested drug was lost through the patient vomiting.

Second, the ingested dosage might have been generally adequate but insufficient for a particularly corpulent patient with a fairly high para-

sitaemia, whether the plasmodium involved is *P. falciparum* or any other species.

Finally, and especially with regard to *P. falciparum*, it is possible that the usual dosage of an effective drug was inadequate because the strain involved exhibits specific resistance to the compound or group of compounds used for treatment. In this case the treatment must be carried out by alternative drugs to which the relevant strain is susceptible. Details of such procedure are given on pp. 131-132.

However, recrudescent infections with *P. falciparum* or other species of plasmodia not truly resistant to quinine or 4-aminoquinolines given at a dose appropriate for the acute attack may be prevented by follow-up treatment at suppressive dosage with any good schizontocidal drug. A regimen consisting of chloroquine or amodiaquine (300 mg base once a week) for 4–8 weeks should be adequate in most circumstances, although in areas of known resistance to 4-aminoquinolines an alternative short-term suppressive course using a combination of a sulfonamide with an antifolic compound may be of value.

Where the local malaria eradication programme has achieved, or is close to achieving, interruption of transmission of the infection, it may be desirable to prevent the reintroduction of malaria by the earliest possible elimination of gametocytes of *P. falciparum* that are not affected by schizontocidal drugs such as the 4-aminoquinolines or quinine. For this particular purpose a single dose of 45 mg primaquine is sufficient to destroy falciparum gametocytes present in the blood. As an alternative, 2 doses of 25 mg pyrimethamine given at an interval of 1 week may produce the desired sporontocidal effect and prevent transmission by the mosquito, provided that the parasite strain is not resistant to the drug.

In true relapsing infections caused mainly by *P. vivax* but also by *P. ovale*, a proportion of cases will show a recurrence of the symptoms and of parasitaemia at various intervals of weeks or months after an apparent cure of the acute attack by schizontocidal compounds. In this case radical cure requires a course of treatment with one of the generally used 8-aminoquinolines, of which primaquine is the best known, more active than any other drug of this series, and least toxic. The usually recommended course is 15 mg primaquine daily for 14 days. Reports on large numbers of patients treated with this regimen, even in areas where G6PD deficiency is quite common, indicate that this regimen is generally well tolerated and that haemolysis, when it occurs, is mild and self-limiting. A reduction of the duration of this treatment to 5–7 days decreases substantially the proportion of radical cures achieved. If the daily dosage is increased to 30 mg a day the results are more satisfactory since radical cure is a function of the total dose of this drug. However, adverse effects (abdominal cramps, diarrhoea and other gastrointestinal symptoms) occur more commonly. For this reason, and especially because of the possibility of haemolytic effects in patients with G6PD deficiency, the

increased daily dosage of primaquine should be given only under medical supervision.[2]

There is evidence that some strains of *P. vivax*, especially those from the south-west Pacific, require a daily dose of 22.5–30 mg for 14 days. Obviously this regimen must be closely supervised to avoid any serious adverse effects.

In these cases equally good results may be obtained by giving 45 mg primaquine and 600 mg chloroquine once a week over a period of 8 weeks, even if this course may pose some operational difficulties.

It is doubtful if radical treatment of vivax malaria is necessary if the patient lives in an endemic area where transmission of the infection continues and reinfection is likely. It may be preferable to give the usual schizontocidal treatment for the primary attack and to warn the patient of a probable relapse (which cannot be distinguished from a reinfection) and to treat the renewed symptoms either with another schizontocidal regimen or with a combination of it with an 8-aminoquinoline at the usual dosage and course. If the patient is already on a suppressive (prophylactic) regimen, the relapse is not likely to occur. However, in cases of malaria imported into a non-malarious area, a course of radical treatment may be advisable. This is particularly so for patients who may have been infected with 2 species of plasmodia and in whom a latent vivax infection may accompany an overt attack of falciparum malaria.

Treatment of Severe Infection

As mentioned before, any infection with *P. falciparum* in a nonimmune individual is a potential medical emergency. Immediate treatment must never be delayed, irrespective of whether the patient shows any symptoms of involvement of the central nervous system (cerebral malaria) or other organs (severe headache, prostration, delirium, hyperthermia, collapse, jaundice, diarrhoea) or of whether he does not appear to be gravely ill but blood examination reveals a high degree of parasitaemia, with or without the presence of maturing schizonts of *P. falciparum*. The clinical picture in this infection may change with dramatic speed, and the survival of the patient depends on the combined skills of the physician and the nursing staff, guided by a competent microscopist. The complexity of the clinical picture of "pernicious malaria" calls for experienced assessment of every individual case and management in hospitals equipped with the necessary haematological

[2] In France a combination of two 8-aminoquinolines, under the name of Rhodopraequine, was and is still occasionally used for the radical cure of relapsing malaria. The regimen adopted for this drug combination, containing 10 mg of each compound, was 3 tablets a day for the first 3 days, followed by 3 tablets once a week during the next 6 weeks. This course was recommended after conventional schizontocidal treatment. As an alternative, a combination of Rhodopraequine and chloroquine under the name of Prenaline, was and is still occasionally used at the above dosage.

and biochemical laboratories. This is not always possible, however, and it must be said that equally good results of treatment have been seen wherever good clinical judgement and some essential facilities have been available.

Much information on the course of the disease and on its response to treatment can be obtained from the parasite count and the total red cell count. The simplest way of counting the percentage of infected erythrocytes is on a thin blood film. At least 1000 erythrocytes should be counted, the number of infected cells and (if possible) the number of parasites being recorded, because infection of one cell by several parasites is common. Progress can be assessed by continuing to follow up the parasitaemia and haematological indices for 4–5 days after the beginning of treatment. Not uncommonly the results of blood examination provide a better measure of the severity of the disease than the clinical picture. Any patient with 2 % or more of his erythrocytes invaded by *P. falciparum* should be regarded as seriously ill. A coexisting high parasite count (over 100 000 per mm^3) with a low total erythrocyte count (less than 2 million per mm^3) is a danger sign calling for blood transfusion.

The origin of the malaria infection should be ascertained whenever possible in order to prepare for the action needed if a strain of *P. falciparum* resistant to the 4-aminoquinolines is present.

Whether specific antimalaria treatment should start at once or follow other emergency measures depends on the state of the patient. If the latter reaches the hospital with dangerous symptoms, measures to counteract them should be instituted immediately, and it must be remembered that intravenous quinine is a life-saving drug.

General treatment

In the shock syndrome associated with hypovolaemia intravenous administration of fluids is the first line of treatment. Isotonic saline with glucose has traditionally been used for this purpose, but today low-viscosity Dextran 40, which remains longer in the circulation, is preferable. Dextran is administered as a 10 % solution in physiological saline with glucose. An intravenous transfusion of 500 ml expands the blood volume by about 3 times and also has an antithrombotic action. If oliguria persists after the transfusion, there is a danger that additional amounts of fluid will overexpand the plasma volume and cause pulmonary oedema. The aim of judicious treatment is to re-establish an adequate circulating volume without upsetting the electrolyte balance.

A fluid chart should be started and fluids given by mouth as soon as possible. If fluid can only be given intravenously the daily requirement of an adult patient of average weight is about 2.5 l.

The place of sympathicomimetic agents (e.g. vasoconstrictors such as isoprenaline sulfate) in the treatment of shock depends on the experience and views of the physician. Since patients in a state of shock are prone to various pulmonary disorders including oedema, oxygen therapy by nasal catheter may be indicated.

As mentioned before, a high parasite density in falciparum infections together with an erythrocyte count below 2 million per mm^3 or a haematocrit level below 30 % is an indication for blood transfusion. Careful cross-matching of blood is essential.

When patients are convulsed or restless and anxious, free use should be made of sedatives. Morphine should be avoided, thiopental sodium, sodium chlorpromazine or paraldehyde being preferable.

Hyperthermia may be dealt with by tepid sponging and exposure to cool air, with periodical checking of the patient's rectal temperature.

Skilled and attentive nursing greatly improves the chances of recovery. The hourly temperature, pulse rate, respiratory rate and blood pressure should be recorded, since cardiovascular collapse and respiratory depression can occur. Blood samples should be taken once a day for the estimation of the bilirubin, the blood urea, the nitrogen concentration, the parasite count and haematological indices. Detailed medical and nursing records should be kept. Blood urea and electrolyte estimations must be determined to give an idea of the renal involvement. In prostrate patients an indwelling sterile catheter should be inserted to collect urine for the analysis of the specific gravity, the protein, the cellular deposits and the haemoglobin breakdown products. The fluid intake and the volume of urine and vomit should be measured and the patient should be weighed once daily. When renal failure occurs, haemodialysis or peritoneal dialysis should not be delayed in the presence of oliguria or anuria and a rapidly rising blood urea level (over 16 mmol/l).[3]

[3] A brief description of the technique of peritoneal dialysis may not be out of place. A local anaesthetic is injected into the lower central portion of the abdominal wall and a wide-bore needle introduced into the peritoneal cavity. One of the following two sterile fluids is then slowly injected:

Sodium lactate	5.0 g
Sodium chloride	5.6 g
Magnesium chloride	0.15 g
Calcium chloride	0.39 g
Dextrose	13.6 g
Distilled water	1 litre

or

Sodium acetate hydrate	4.7 g
Sodium chloride	5.0 g
Calcium chloride	0.32 g
Magnesium chloride	0.15 g
Anhydrous dextrose	17.0 g
Distilled water	1 litre

Sodium depletion from vomiting and diarrhoea is not uncommon. Acidosis should be corrected with intravenous sodium bicarbonate. In oliguric or overhydrated patients a diuretic such as frusemide in doses up to 500 mg a day may be tried, but this is not without some risk of toxic effect. Mannitol has been used in some cases. In the event of hepatic failure, phytomenadione (vitamin K) injections may be given.

The value of heparin, advocated if signs of disseminated intravascular coagulation appear, is controversial and more recent opinion sees little advantage in using it. Other tentative treatments (e.g. urea injections) have not been generally endorsed.

Specific treatment

This should start as soon as life-threatening cardiovascular, pulmonary or cerebral signs and symptoms have been dealt with. Often nonspecific intervention and antimalarial therapy can be combined.

The most effective drug is quinine, and its supremacy over chloroquine has been restored in view of the existence of chloroquine-resistant strains of *P. falciparum* in some parts of the world. It is obvious that many signs and symptoms of severe infection (coma, convulsions, vomiting, diarrhoea) require that the drug should be given by injection. The consensus of experienced medical opinion favours the intravenous administration of quinine, but conservative physicians in some countries warn against the risk of a sudden fall in blood pressure or of quinine hypersensitivity and advocate intramuscular injection.

The principle in the intravenous administration of quinine is high dilution and very slow infusion. Thus the best method is to give the drug in an intravenous drip composed of 500 ml of glucose saline, plasma or dextran solution. The initial adult dose of 500–1000 mg of quinine hydrochloride or dihydrochloride should thus be given over a period of 1 - 2 hours. If necessary this can be repeated within 24 hours, but the total dose of quinine over that period must not exceed 2000 mg.

If there are no facilities for intravenous infusion smaller intravenous doses of quinine (250–500 mg) can be given by syringe in 20 ml glucose saline; a fine needle should be used and the injection should be given over not less than 10 minutes by the clock. More rapid injection may cause a fall in blood pressure and cardiac arrhythmia.

Potassium chloride 250 mg may be added to each litre of the two solutions if the serum potassium level is low. The second solution may prevent the occurrence of accidental peritonitis better than the first.

For adults 1.5 -2 litres of the solution are injected through the wide-bore needle, which should then be replaced by a narrow sterile catheter with lateral holes. The injected fluid should be withdrawn every 1 –2 hours during the day and replaced by 1 litre of fresh sterile solution. Dialysis should continue for some time even after normal diuresis has started.

Intramuscular injections of quinine are an acceptable alternative (though not without some disadvantage), provided that the solution of quinine hydrochloride is sterile and nearly neutral in reaction, that the site of the injection is well chosen,[4] and that the single dose does not exceed 1000 mg, the total dosage being less than 2000 mg in 24 hours.

Some clinicians believe that in severely ill patients the metabolism of quinine is slow, owing to the impairment of hepatic function. In order to avoid the toxic effects of the drug they advocate the relatively low dose of 10 mg/kg, with an optimum dosage interval of 12 hours. After an initial response to the drug the dose may be increased to 20 mg/kg and even 30 mg/kg if necessary.

The following guidelines for the use of intravenous quinine and fluid infusion have been recommended (Hall, 1976).

Weight of patient	*Daily dose of quinine*	*Daily volume of fluid*	*Number of intravenous infusions*	*Infusion rate*
(kg)	(mg)	(ml)		(ml/h)
5	100	200	1	25
10	200	200	1	50
25	500	500	2	63
50	1000	1000	2	125

Note: Recommended daily intravenous fluid intake 20 ml/kg; 40 ml/kg in small children. Standard infusion time 4 hours to minimize the possible toxic effects of the drug.

Oral treatment must replace parenteral administration as soon as possible.

Chloroquine is as good as quinine for strains of *P. falciparum* susceptible to the 4-aminoquinolines. In severe malaria chloroquine can be given either intravenously or intramuscularly. For intravenous administration the principles mentioned under quinine are equally valid. The single average adult dose is 200–300 mg base in a 4%–5% solution. If intravenous infusions of glucose saline or dextran solutions are indicated, the opportunity may be taken to add chloroquine to the slow drip.

Intramuscular injections of chloroquine have almost the same rapid action as intravenous injections and are preferred, since they are generally well tolerated. Single doses of 300–400 mg base (10 ml of a 5% solution) can be repeated up to a total of 900 mg in 24 hours.[5] Oral therapy should be started as soon as possible.

---

[4] The correct site of injection is 6–7.5 cm below the centre of the iliac crest, deep into the gluteal muscles. Scrupulous care should be taken to assure the sterility of the syringe, needle and solution. Some proprietary formulations of quinine–urethane or quinine–antipyrine cause less pain on injection than quinine hydrochloride. Even then local indurations may occur in some persons and resolve very slowly. It should be remembered that in the presence of severe thrombocytopenia with a platelet count below 20 000 per mm^3 an intramuscular injection may create a large haematoma.

[5] Intramuscular injections of chloroquine can be dangerous when given to children (see pages 133 and 145).

Amodiaquine resembles chloroquine in its antiplasmodial action, though it may be more effective against some strains of *P. falciparum*. In those circumstances it may be an acceptable substitute for oral medication at the dosage mentioned previously. However, as parenteral preparations of amodiaquine are not available, amopyroquine (an analogue of amodiaquine) may be used for intramuscular administration at the same dosage.

Mepacrine will be mentioned only briefly, since this is an obsolete drug. Intramuscular injections of the soluble methanesulfonate (mepacrine mesylate) are effective, though the drug is more toxic than chloroquine, especially in children. The single dose for an intramuscular injection is 300 mg. Intravenous injection of this drug is dangerous and must never be attempted.

Other drugs or drug combinations used specifically against strains of *P. falciparum* resistant to the 4-aminoquinolines are described below.

Treatment of Malaria Resistant to 4-Aminoquinolines

When resistance to chloroquine in infections attributable to *P. falciparum* is suspected in a patient from one of the main areas of the present distribution of these strains, recourse must be made to alternative compounds. Such compounds may also be needed when there has been a clear recrudescence of parasitaemia and clinical symptoms after an adequate course of chloroquine.

In these circumstances quinine alone or in combination with other drugs has been sometimes used, e.g. a regimen consisting of 600 mg quinine 3 times daily for 1 week, followed by 50 mg pyrimethamine daily for 2 days and 25 mg dapsone daily for 3 weeks.

However, recent experience has shown that the best results are obtained with a short course of quinine (600 mg, 3 times daily, for 2 or 3 days) followed on the third day by 3 tablets of a combination of long-acting sulfonamides with pyrimethamine (in a ratio of 20:1). The sulfonamide–pyrimethamine combination may also be given on the first day of the quinine course. In severe malaria, quinine given intravenously on the first day has a more rapid effect.

Among the combinations of sulfonamides with antifolic compounds the following have been used with satisfactory results:

(1) sulfadoxine	1500 mg	to be given as a single dose
pyrimethamine	75 mg	

(this drug combination is also available under the trade name of Fansidar in tablets containing 500 mg sulfadoxine and 25 mg pyrimethamine)

(2) sulfalene	1500 mg	single dose
pyrimethamine	75 mg	

(this drug is available under the trade name of Metakelfin in tablets containing 500 mg sulfalene and 25 mg pyrimethamine).

The combination of sulfadoxine with pyrimethamine (Fansidar) seems to have achieved wide acceptance at the present time because of its simplicity, reliability and absence of adverse effects. However, this drug is not available in all countries. An injectable preparation of Fansidar has been introduced and appears to be of value; it consists of 2.5-ml ampoules, each containing 500 mg sulfadoxine and 25 mg pyrimethamine. The adult dosage is 2 ampoules, to be given in a single dose according to age group and body weight, either by intramuscular injection or by slow intravenous drip. However, it would be a mistake to consider injectable Fansidar (which is not a rapidly acting drug) as a substitute for quinine in severe cases of malaria. Among the combinations of short-acting sulfonamides (such as sulfamethoxazole) with trimethoprim, at a respective ratio of 5 to 1, co-trimoxazole has gained wide popularity for the treatment of many bacterial infections. Several attempts have been made to assess the value of these drug combinations in the treatment of malaria. It appears that, given in dosages of 10–16 tablets[6] over 3–5 days to semi-immune individuals, a considerable reduction in the parasitaemia has been achieved. However, the consensus of expert opinion is that these drug combinations are not suitable for the treatment of acute malaria since other and better drugs are available

P. falciparum infections resistant to chloroquine and sulfonamide/pyrimethamine combinations may be treated with quinine (adult dose 3 × 600 mg per day for 5–7 days) simultaneously with or followed by tetracycline (adult dose 4 × 250 mg per day for 7 days).

Another important advance in the treatment of falciparum malaria resistant to 4-aminoquinolines is the introduction, so far only on a limited scale, of *mefloquine*. This new drug has been given (by some authors after an initial short treatment with oral or parenteral quinine) at the adult dose of 1.0–1.5 g (4–6 tablets of 250 mg each). The results of this treatment have been satisfactory.

Mefloquine appears to cause occasional minor adverse effects on the gastrointestinal tract, especially in febrile patients.

[6] The usual content of a tablet of these combined drugs is 400 mg sulfamethoxazole and 80 mg trimethoprim

Treatment of Malaria in Children

As the diagnosis of acute malaria in a child is subject to even more vagaries than in an adult, great watchfulness should be maintained whenever there is any possibility that the symptoms may be attributable to a plasmodial infection Vivax malaria may occasionally produce an alarming clinical picture in children, but usually it is falciparum infection that gradually or suddenly develops into a medical emergency A severe falciparum infection is present if convulsions, stupor, collapse, copious vomiting and diarrhoea, anaemia, or jaundice are seen, or if the parasite count indicates that over 2% of the red blood cells are infected

Treatment of malaria in children is essentially the same as in adults, with the proviso that some drugs (e g quinine) are relatively better tolerated by children, while other drugs (e g intramuscular injections of chloroquine) call for greater caution (see below)

Coma and severe vomiting may make oral therapy impossible and in all such cases where speed is important parenteral administration is indicated, although drugs given orally are by far the safest In older children cautious intravenous administration is permissible Quinine and chloroquine are the only two drugs suitable for the intravenous route, either should be given in high dilution and very slowly, preferably by infusion or by continuous intravenous drip in glucose saline or plasma The average dose of quinine is 5 10 mg kg body weight This dose should be repeated, if necessary, 6 12 hours later, but the total dose over 24 hours should not exceed 20 mg kg body weight at a concentration of 1g l infused over 2 4 hours The single dose of intravenous chloroquine averages 5 mg base per kg body weight, to be repeated in 6 8 hours if necessary Alternatively a higher dose corresponding to 7 mg base per kg body weight can be given as a continuous intravenous drip over 24 hours

Quinine or chloroquine can all be given intramuscularly although this method of administration may also carry some risk Intramuscular injection of quinine may produce some tissue necrosis and cause a deep abscess, unless the technique of injection is faultless and the suitability and sterility of the solution are perfect If financial circumstances permit, proprietary ampoules of quinine solutions are therefore to be preferred to locally made preparations of the drug Single doses of intramuscular quinine should not exceed 15 mg kg body weight Chloroquine injections are effective, but in infants and children may cause epileptiform convulsions and circulatory collapse which might be fatal or cause permanent damage to the nervous system Parenteral administration of chloroquine in infants and children is therefore contraindicated

For fear of side effects if chloroquine is given intravenously or intramuscularly, some physicians advocate the subcutaneous route This practice is not generally advisable since local complications such as inflam-

mation and abscess formation may follow and the specific effect on severe malaria is delayed

It is always safer to inject a given amount of chloroquine in two divided doses separated by an interval of 1–2 hours For parenteral treatment of chloroquine-resistant falciparum malaria sulfadoxine with pyrimethamine (Fansidar) solution for injections (containing 200 mg of sulfadoxine and 10 mg pyrimethamine per 1 ml solution) can be given Intramuscular injection or intravenous slow drip of this compound requires the following single dosage

0–4 years	0 5 1 5 ml ($\frac{1}{4}$ $\frac{3}{4}$ ampoule)
5–8 years	1 5–2 ml ($\frac{3}{4}$ – 1 ampoule)
9 14 years	2–3 ml (1–1½ ampoule)

For rapid action quinine is preferable, injected slowly and with care

The routine administration of antimalarial injections in all cases of fever in children, irrespective of whether a blood slide is examined or not, must be sternly condemned In every case oral treatment is the safest and best and should be resumed as early as possible The bitter taste of drugs can be disguised if the crushed tablet is mixed with a spoonful of jam or thick syrup If tablets are administered the child should be observed for half an hour in case vomiting occurs Should this happen the same dose must be repeated For the oral treatment of falciparum infections resistant to the 4-aminoquinolines quinine may be given at the appropriate dosage for 10–14 days, in addition pyrimethamine (at a dosage proportional to the adult dose of 25–50 mg daily) can be given for the first 3 days A treatment to be preferred is a combination of sulfadoxine (1 0 g) and pyrimethamine (50 mg) in single or divided dose over 1–2 days Some authors believe that this should be preceded or followed by 2–3 days of an appropriate dosage of quinine These adult doses must be adjusted to the paediatric dosage

As with other drugs, there is no simple way of calculating the dose of antimalarials for infants and children Fever, acidosis, malnutrition, and dehydration affect the metabolism of the drug Body weight is probably the best and simplest guide to the dosage in children, but it must be used with common sense and in the light of other factors such as the severity of the clinical symptoms or the degree of parasitaemia Table 8 gives a guide to the dosage used for nonimmune children An undersized child of any age group should preferably be given the dosage corresponding to the next lower age group and an oversized child the dosage corresponding to the next higher age group In endemic malarious areas, older children who have experienced previous attacks of malaria develop a considerable degree of tolerance to the disease and respond well to a single dose of 1–2 tablets of any schizontocidal drug Although many younger children and infants in the endemic areas may respond similarly, it should never be forgotten that the host-parasite relationship is unstable in infancy and early childhood and that severe

TABLE 8 DOSAGE OF ANTIMALARIAL DRUGS FOR ORAL TREATMENT OF MODERATELY SEVERE MALARIA IN NONIMMUNE CHILDREN ACCORDING TO AGE

Drug	Up to 1 year $\frac{1}{10}$–$\frac{1}{8}$ of adult dose	1–3 years $\frac{1}{4}$–$\frac{1}{3}$ of adult dose	4–6 years $\frac{1}{2}$ of adult dose	7–11 years $\frac{1}{2}$–$\frac{3}{4}$ of adult dose	12–15 years $\frac{3}{4}$–1 of adult dose	Regimen
Quinine	100–200 mg	200–300 mg	300–500 mg	500–1000 mg	1000–2000 mg	Daily dose to be divided into 2–3 parts continued for 7–10 days
Chloroquine	(i) 75 mg ($\frac{1}{2}$ tablet) (ii) 75 mg ($\frac{1}{2}$ tablet (iii) 37.5 mg ($\frac{1}{4}$ tablet)	(i) 150 mg (1 tablet) (ii) 113 mg ($\frac{3}{4}$ tablet (iii) 75 mg ($\frac{1}{2}$ tablet)	(i) 300 mg (2 tablets) (ii) 150 mg (1 tablet) (iii) 75 mg ($\frac{1}{2}$ tablet)	(i) 300 mg (2 tablets) (ii) 150 mg (1 tablet) (iii) 150 mg (1 tablet)	(i) 450–600 mg (3–4 tablets) (ii) 225–300 mg (1$\frac{1}{2}$–2 tabs) (iii) 150–300 mg (1–2 tabs)	(i) Loading dose (ii) Second dose following loading dose after 6 hours (iii) Daily dose for the next 2–4 days
Amodiaquine	(i) 50 mg (ii) 50 mg	(i) 100 mg (ii) 50 mg	(i) 150 mg (ii) 100 mg	(i) 200–300 mg (ii) 150–200 mg	(i) 400–600 mg (ii) 250–400 mg	(i) Dose for the first day (ii) Daily dose for the next 2–4 days
Sulfadoxine + pyrimethamine (Fansidar)	250 mg + 12.5 mg ($\frac{1}{2}$ tablet)		500 mg + 25 mg (1 tablet)		1000 mg + 50 mg (2 tablets)	Single dose
Sulfalene + pyrimethamine (Metakelfin)	Same as above					Single dose

Note The dosage of chloroquine and amodiaquine is expressed in terms of base. The upper limit of the adolescent dose constitutes the generally adopted adult dose. Dosages of chloroquine are adjusted for fractional use of the common formulation of the drug containing 150 mg of base per tablet. In some countries chloroquine is formulated in tablets of 100 mg base. Mepacrine now considered an obsolete drug has not been included in the table.

infections and complications may occur at any time and require vigorous treatment at full dosage.

Some clinicians in France and other French-speaking countries have good results with chloroquine in suppositories for the treatment of children. The dosage is twice that given orally. This treatment may be of value in some cases, though its action is relatively slow.

In addition to the specific treatment of malaria in children, general treatment and excellent nursing are of primary importance. In convalescence a high protein and high vitamin diet is indicated, supplemented if necessary with iron preparations.

Treatment of relapsing and drug-resistant malaria in children is not greatly different from that in adults. Falciparum infections may occasionally recur and require the same treatment as the acute attack. Vivax or quartan malaria has a general tendency to relapse, the latent period and the number of relapses depending on the strain of the parasite and previous treatment. In view of the relative toxicity of the 8-aminoquinolines, it is preferable to treat relapsing attacks in small children by chloroquine or any other good schizontocidal drug, with the addition of a prophylactic drug such as pyrimethamine (12.5 mg base) once a week between the attacks. For older children the best treatment of the primary attack appears to be with chloroquine, followed by the administration of proguanil or pyrimethamine for 2–3 months. Relapses of vivax malaria may then be treated with a combination of chloroquine (or amodiaquine) and, afterwards, primaquine. The dose of primaquine for a child aged 4–8 years is 7.5 mg base daily for 7–10 days. Patients on such a primaquine regimen should be under medical supervision for the early detection of toxic symptoms.

Treatment of Malaria in Pregnancy

Malaria infections generally, and falciparum infections especially, occurring in pregnant women deserve special mention for a number of reasons. In the late stages of pregnancy mothers in highly endemic areas lose some of their acquired immunity and may suffer from more severe attacks. One of the features of falciparum malaria in these parts of the world is a high frequency of infection of the placenta. The concentration of parasites in the placenta has an effect on the newborn baby, which is often underweight and less able to thrive. Neonatal and infant mortality is much higher in these children. Malaria may occasionally be transmitted from the mother to the child across the placenta, but this occurs more commonly in nonimmune women.

Another aspect of malaria in pregnancy in highly endemic areas is severe anaemia, which is a common cause of mortality among women in India and tropical Africa. The pathogenesis of haemolytic and megaloblastic anaemia of pregnancy is uncertain but it is probably due to a combination of nutritional

and parasitological causes particularly common in primiparous women. There is mixed iron deficiency and folic acid deficiency, the haemolytic consequences of falciparum infection are greater than would be expected from the degree of parasitaemia Loss of blood at delivery is an additional important factor The chances of stillbirth or of complications of delivery are enhanced by the infection of the mother and contribute to the high maternal mortality in tropical malarious areas

Malaria in a pregnant woman must be regarded seriously and treated accordingly Antimalarial drugs should be given during pregnancy and the puerperium The choice is between causal prophylactic drugs (proguanil, pyrimethamine) and suppressives (chloroquine, amodiaquine) and depends on the endemicity of the area, the presence or absence of resistant strains of plasmodia, and the health conditions of the pregnant woman Given in the usual preventive doses (Table 9), the commonly used drugs have no abortive or teratogenic effects

TABLE 9 DOSAGE OF ANTIMALARIALS COMMONLY USED FOR INDIVIDUAL PROTECTION IN AREAS WHERE PARASITES ARE SENSITIVE TO THE PARTICULAR DRUG

Drug	Frequency of administration	Dose (mg) according to age (years)					
		over 16	11 16	7 10	4 6	1 3	under 1
Proguanil (salt)	daily	100	100	75	50	50	25
Pyrimethamine	once weekly	25	25	18	12	6	
Amodiaquine (base)	weekly[2]	300	225	150	100	75	25
Chloroquine (base)	weekly[3]	300	225	150	100	75	25

[1] On the same day every week e g Sunday or Friday

[2] Proprietary tablets are available containing 100 mg amodiaquine base

[3] On the same day every week or at half the dosage twice a week

Note In some parts of the world where the transmission of malaria is intense the dosage of proguanil may be doubled (2 tablets or 200 mg daily) for limited periods

In French speaking parts of tropical Africa the usual chloroqu.. ie prophylaxis regimen for adults is 100 mg (base) daily or for 6 days a week during the transmission period

In the treatment of acute malaria in pregnant women the usual drugs and dosages apply However, in view of the haematological effects of pregnancy in highly endemic areas it is advisable to administer folic acid at a dosage of 5 mg daily to prevent folate deficiency (which may be aggravated by pyrimethamine) If there is a deficiency of vitamin B12 it should be remedied by a daily dosage of 50 μg Iron preparations are indicated and oral or injectable forms commonly used In severe anaemia blood transfusion may be needed as a life-saving measure Proper nutrition with green leafy vegetables and protein is of great importance Primaquine is contraindicated during pregnancy

Treatment of Malaria in Semi-Immune Patients

As mentioned in Chapter 2, the repeated infections with many species and strains of malaria parasites prevalent in highly endemic malarious areas (e.g.

tropical Africa) eventually produce a degree of immune response that modifies, often considerably, the symptoms of clinical attack. This is particularly obvious in adults, less so in adolescents and schoolchildren and of little evidence in very young age groups with the exception of infants up to the age of about 6 months, who are partly protected by transplacentally derived maternal antibodies.

The immunity may be related to one prevalent species or strain of malaria parasite or may partially protect from the effects of most species of plasmodia. It usually regresses when the person concerned has been outside the endemic area for several years. If this occurs reinfection may be severe and the patient should be treated in the same way as a nonimmune individual.

On the other hand, infection of the semi-immune, and especially of adults residing in endemic areas, responds rapidly and satisfactorily to the standard chemotherapeutic regimen at a reduced dosage. It should be remembered, however, that the level of immunity decreases with time in an area where the transmission of malaria has been sufficiently reduced by intensive control measures. Relaxation of these measures may result in a resurgence of transmission among the local population, who may have partially lost their immunity. This is particularly important with regard to infants and small children.

As antifolic drugs are slow schizontocides and the areas of resistance to them are not well known, the appropriate treatment is with the 4-aminoquinolines. For adults a single dose of 600 mg chloroquine base or 600 - 800 mg amodiaquine base often suffices to relieve the symptoms and eliminate the parasites. Quinine (1.5 - 2 g as a total dose) or mepacrine (300 - 600 mg) may be used as an alternative. Proguanil (300 - 500 mg) or pyrimethamine (50 mg) may be given if no other drugs are available Sulfonamides with pyrimethamine should be reserved for areas where resistance to the 4-aminoquinolines has been confirmed.

Malarial Haemoglobinuria (Blackwater Fever) and its Treatment

This syndrome, involving acute haemolysis and subsequent haemoglobinuria, is associated with endemic falciparum malaria. The condition is seen mainly in nonimmune persons who have had a history of repeated clinical attacks, inadequately treated or suppressed by quinine. It was formerly common among American, Asian and European adults who came from nonmalarious areas to regions of hyperendemic malaria; their children, however, were less frequently attacked.

It was rare among indigenous adults who had grown up in areas of hyperendemic malaria, but it occurred at times when they moved from one area to another. It occasionally occurred among indigenous children,

especially if they took quinine irregularly to treat or suppress malaria. In children the attack is usually less severe than in adults

It is probable that the disease is caused by a state of hypersensitivity attributable to the presence of an incompletely suppressed falciparum infection In this state any unusual factor (chill, exhaustion, injury, etc) may cause a sudden intravascular haemolysis, possibly owing to auto-antibodies produced in response to some immunological changes in the parasitized red blood cells. The intravascular haemolysis is often so severe that the red cell count may fall by 20–50 % within a period of 24 hours Methaemalbumin and haemoglobin appear in the plasma, the liver attempts to deal with the products of haemolysis, and the serum bilirubin increases, giving rise at first to an indirect van den Bergh reaction With increasing damage to the hepatic parenchyma frank jaundice appears Patients dying at this stage present signs of hepatic lesions, circulatory shock, and heart failure

The early clinical symptoms are very similar to those of a severe paroxysm of falciparum malaria, until the haemolytic phase manifests itself by the passage of small amounts of dark red or black urine of high albumin content containing a copious deposit of hyaline and granular casts, epithelium and blood pigments In severe cases this is usually preceded by a state of shock with a sudden drop in the temperature In milder cases the haemolytic crisis abates and the urine, passed in increasing amounts, becomes clear after a few hours or a day

Severe cases of blackwater fever show more or less pronounced symptoms of hepatic involvement with liver enlargement, nausea, vomiting, diarrhoea and jaundice Pallor is later masked by the jaundice, the pulse rate is always much increased and there is a fall of blood pressure in those who are severely shocked There is a decreased urinary output and increasing anaemia At this stage the general condition, the red cell count, the volume and character of the urine and the fluid chart recording the intake and output suggest the prognosis and indicate the treatment If haemolysis ceases and hydration is adequate, shock rapidly subsides and the urinary output returns to normal

The consequences of recurring massive intravascular haemolysis are particularly obvious in patients who remain in a critical condition for several days Their renal failure is not due to the mechanical blockage of tubules by haemoglobin pigments precipitated by acid urine but mainly to local ischaemia with resulting damage to the nephron If severe renal changes occur complete anuria ensues, vomiting and drowsiness become marked, the blood urea rises to very high figures and uraemia leads to a fatal issue

Treatment Rest in bed is essential, in remote areas home nursing is preferable to the hazard of moving the patient to hospital Some patients have only one brief episode of haemolysis without any complications. Severe cases require restoration of the red cell and blood volume by a blood transfusion, but careful matching is essential since the agglutinin pattern is disturbed in these cases. Both the cells and the plasma must be cross-matched for every

blood unit transfused. Less severe cases may require only the correction of the dehydration and the loss of electrolytes; vomiting is an important factor in producing these complications and may require the parenteral administration of saline and glucose. Excessive transfusion and overloading of the circulation with plasma or intravenous infusions must be avoided Prednisolone phosphate (40–60 mg) given intramuscularly may help to contain the haemolysis, and in some cases the response is rapid.

Parasitaemia in cases of massive haemolysis is scanty or, more often, absent. However, if malaria parasites are found, chloroquine or amodiaquine is the obvious choice, though quinine, administered cautiously, need not be excluded. In practice the patient is in acute renal failure when the daily amount of urine is less than 400 ml and the plasma urea concentration is over 16 mmol/l. A 24-hour record of fluid input and output should be kept and the specific gravity of urine must be measured in every sample. If a sample of each specimen of urine is kept, the series will give an indication of the patient's progress. The plasma urea concentration must be monitored and, if possible, the plasma concentration of the antimalarial drug also When the plasma urea concentration is nearing 33 mmol/l renal dialysis should be instituted. If this is not feasible, peritoneal dialysis should be carried out. Various methods of treatment such as snake antivenin, ascorbic acid in large doses and the injection of procaine hydrochloride into the renal pedicles have been tried, with uncertain results. Diuretics and alkalinization of the blood may do more harm than good. When the patient recovers from an attack of blackwater fever, further curative antimalarial treatment and continued prophylaxis may prevent recrudescence.

Adverse Effects of Antimalarial Drugs[7]

Quinine

Serious adverse effects from the clinical use of quinine are very rare when the plasma concentration of quinine is less than 5 mg/l. Among side effects seen occasionally when the daily dosage is 600–1500 mg are giddiness, headache, impaired hearing, ringing in the ears (tinnitus) and nausea. Tremors, depression and blurred vision may occur during the first days of administration but these symptoms (known as cinchonism) are generally transient and disappear without trace. In some individuals, especially women, they may be so unpleasant that an alternative treatment must be prescribed.

Children seem to tolerate the proportional dosage of quinine by oral administration relatively better than adults.

[7] The most important points concerning the tolerance towards, toxicity of and contraindications to commonly used antimalarial compounds have been given in Chapter 3. The present section is an expansion of certain relevant aspects.

There is no evidence whatsoever that quinine causes sterility in the female or impairs libido in the male. It does not affect pregnancy unless taken deliberately in toxic doses, and even then the effects are attributable to the general poisoning and not to a specific action on the uterus.

Idiosyncrasy to quinine occurs, but is rare. Urticarial or erythematous rashes with intense itching, subcutaneous or submucous haemorrhages, oedema of the eyelids, mucous membrane or lungs, and even collapse have been known to follow a single dose. Fever unrelated to parasitaemia may develop during the course of quinine therapy if it is prolonged. Such fever usually occurs after about 1 week from the beginning of treatment and decreases within 1–2 days following withdrawal of the drug. Haemoglobinuria and anuria may be caused by quinine (as distinct from the malaria infection itself) in rare cases of idiosyncrasy or overdosage.

When the daily dosage of quinine is excessive or when accidental poisoning takes place the symptoms described above are more pronounced. The most alarming effect is amblyopia, occurring suddenly within an hour of taking a large dose; at times only a contraction of the field of vision occurs. In most cases of quinine amblyopia recovery is the rule, but improvement may be very slow. Serious impairment of hearing may also occur. Deafness has been observed in babies born from mothers who have taken toxic doses of quinine.

Poisoning by doses of over 3 g should cause serious concern, although recovery from a dose as high as 10 g or more has been described.

Among serious toxic effects from careless or excessive parenteral administration of quinine those related to rapid intravenous injection are of particular importance. Intravenous injection given too quickly may cause a fall in blood pressure, sudden heart block, ventricular fibrillation and death.

Treatment of acute quinine poisoning consists of counteracting the fall in the blood pressure by analeptic drugs such as the adrenergic amines. For quinine amblyopia vasodilators such as amyl nitrate or nicotinic acid derivatives have been recommended. Idiosyncrasy to quinine, if suspected, can be confirmed or otherwise by appropriate skin tests.

For the treatment of acute oral poisoning by quinine gastric lavage should be carried out using a solution of magnesium sulfate, some of which should be left in the stomach.[8] Shock, hypotension, central depression, respiratory and cardiac arrest may require artificial respiration, ephedrine, amphetamine and intravenous fluids. If the patient can be kept alive for 24 hours a fatal outcome is generally unlikely. For the treatment of ocular disturbances various substances have been proposed; nicotinic acid 50–200 mg, papaverine 30–60

[8] After initial aspiration of stomach contents the lavage should be carried out using 250 ml at a time of a solution of 100 g magnesium sulfate per litre of water, alternatingly instilling and aspirating the fluid. Quantities in excess of 250 ml may have the undesirable effect of forcing the pylorus open. It is recommended to leave a cathartic dose of 15–30 g magnesium sulfate in the stomach (some 150–250 ml of the solution).

mg and sodium nitrite 100 mg have been given parenterally, sometimes with dramatic results. These substances should not be given while the patient is in shock. Amyl nitrite by inhalation and atropine by subcutaneous injection have also been used. Bilateral block of the stellate ganglion has been successful, but this intervention is one for a specialist.

There is good evidence that, in persons infected with *P. falciparum* and exposed for some time to repeated reinfection, large or even small doses of quinine may precipitate an attack of haemoglobinuria. The mechanism of the haemolytic syndrome is obscure, though it is probably due to an autoimmune process somehow triggered by quinine.

Primaquine and other 8-aminoquinolines

Soon after the discovery of pamaquine (the first compound of this series) adverse reactions to this drug were reported. They include nausea, vomiting, stomach pains, cyanosis, dizziness, haemolytic anaemia and, when high doses were given, agranulocytosis.

These effects are much less common following the administration of primaquine and of other modern representatives of the 8-aminoquinoline series. The general clinical experience with the 8-aminoquinolines has led to the use of relatively small doses of primaquine in man, the results showing that a daily adult oral dose of 15 mg may be safely given for 2 weeks without medical supervision. Higher doses—30 mg for 2 weeks—can be given to the majority of those patients who are aware of the possible side effects of the drug and able to judge when to stop it. Further studies (by Alving and his group) indicate that the haemolytic effect of primaquine is decreased with intermittent use (at weekly intervals) rather than daily use of the drug. In Thais, for example, a single dose of 45 mg base may result in the haemolysis of up to 20% of the circulating red cells, but these are replaced within the next few days by younger cells formed in the haemopoietic tissues. Consequently, a single dose of 45 mg once a week is better tolerated than a daily dose of 15 mg primaquine.

The principal toxic effect of primaquine is its haemolytic action on some human erythrocytes, especially in subjects with a deficiency of glucose-6-phosphate dehydrogenase (G6PD), an essential enzyme controlling the pentose phosphate pathway of glucose metabolism. Such deficient cells are susceptible to oxidative damage and are more fragile. This hereditary enzymatic defect is found in about 10% of American Negroes and up to 20% of Negroes in West Africa, but is also common in some other populations in Africa, Asia, southern Europe and the eastern Mediterranean area. It has been stated that about 100 million people are known to have G6PD deficiency.

G6PD deficiency has a sex-linked, partially dominant mode of inheritance. It manifests itself fully in hemizygous males. On the other hand it is only partially manifested in heterozygous females.

There are two variants of the enzyme, one that is fast moving on electrophoresis (A), and one that is slow moving (B). It is the first one that is deficient in primaquine-sensitive subjects, principally in Negroes; type B enzyme deficiency is found in some Negroes and in Caucasians. Clinical haemolytic manifestations in white children with G6PD deficiency may occur even without drug provocation. In adults manifesting this enzymopathy the haemolytic effect occurs when some drugs are ingested, among which primaquine, the sulfones, sulfonamides, nitrofurans, antipyretics and analgesics are the most common. The haemolytic effect passes through 3 stages: (1) acute haemolysis with a latent phase of 1–3 days followed by a fall in the haemoglobin level and some haemoglobinuria; (2) a recovery phase during which there is an increase in the reticulocytes and haemolysis disappears; (3) an equilibrium phase when, in spite of drug administration, no further haemolysis takes place but there is a shortened survival of erythrocytes and compensation through increased erythropoiesis.

The prognosis of this condition is good and, apart from the avoidance of the drugs that cause it, no other treatment is called for. The probability of cumulative haemolytic reactions in G6PD-deficient subjects taking several drugs (e.g. sulfonamides and antipyretics) should be stressed.

For the treatment of malaria in persons with this enzymopathy a daily dose of 15 mg of primaquine should not be exceeded. Moreover, primaquine should not be given at the conventional dosage together with mepacrine, chloroquine or amodiaquine. Daily urine and blood examinations should be made when monitoring the haemolytic effect. Diagnosis of G6PD deficiency can be made from examination of the blood film, which shows the presence of Heinz bodies during the first phase of haemolysis. The use of a simple screening test based on the reduction of methylene blue is indicated whenever any widespread use of primaquine is planned.[9] Primaquine administration in light-skinned subjects with G6PD deficiency may result in a greater degree of haemolysis than in dark-skinned subjects.

Drug administration should be stopped when a marked darkening of the urine or a sudden decrease in the haemoglobin level occurs. Primaquine is contraindicated in malarious patients suffering from certain systemic diseases, such as rheumatoid arthritis or lupus erythematosus, or in patients receiving drugs depressing bone marrow activity.

For the treatment of primaquine poisoning, whether acute or chronic, folinic acid at a dosage of 10–20 mg daily should be given.

Mepacrine

Only a brief mention of the adverse effects of this drug will be made here, since its use for the prevention or treatment of malaria is now very

[9] See WHO Technical Report Series, No 366, 1967. *Standardization of procedures for the study of glucose-6-phosphate-dehydrogenase deficiency* Report of a WHO Scientific Group

rare, mepacrine having been largely superseded by other less toxic compounds.

When administered as a prophylactic, mepacrine causes yellow discoloration of the skin and, occasionally, dark pigmentation of some mucous membranes. Both in prophylactic and therapeutic use it may cause nausea, vomiting, blurring of vision, abdominal cramps and diarrhoea. There may be various skin lesions such as lichen planus and even exfoliative dermatitis. Aplastic anaemia has been reported on rare occasions. Mental excitation, convulsive seizures, mania or depression have occurred in a number of cases, but have generally been of a transient character.

No close relationship has been observed between the dosage of mepacrine and the occurrence of adverse effects on the central nervous system.

It should be emphasized that intramuscular injection of mepacrine in children is a particularly dangerous practice, leading either to collapse and death or to the lifelong effects of encephalopathy.

Mepacrine is contraindicated when 8-aminoquinolines are being administered at the same time. It is also not advisable to use this drug in neurosyphilitics. This drug may still be used for the treatment of some tapeworm infections and in giardiasis.

Chloroquine and other 4-aminoquinolines

The adverse effects of this series of compounds are known mainly on the basis of experience with chloroquine, the most commonly used. Many studies on experimental animals were carried out before any of the drugs were released for human administration. It appears that differences between them in acute toxicity to animals are not great. Chloroquine seems to be more toxic to dogs than amodiaquine; amopyroquine is less toxic than amodiaquine; and hydroxychloroquine is the least toxic of the 4.

A new series of studies on the long-term toxic effects of these drugs have been carried out more recently, when the value of 4-aminoquinolines at high dosage for the treatment of rheumatoid arthritis was discovered. The results of this research have to a large extent confirmed the findings reported during the treatment of human subjects for malaria.

In man the adverse effects of the 4-aminoquinolines are generally related to the dosage of the drug and its mode of administration. At the conventional adult dosage for the treatment of acute malaria (Table 7, p. 123) or for the suppression of malaria (Table 9, p. 137), the side effects of chloroquine, amodiaquine or hydroxychloroquine are rare and mild. Nausea and vomiting may occur when the drugs are taken on an empty stomach. Pruritus of the palms, soles and scalp has been reported, as also the occasional headache and transient blurring of vision caused by difficulty in accommodation. These symptoms, generally related to the full therapeutic regimen, disappear when the medication is discontinued. Other side effects

include photoallergic dermatitis, pigmentation of the skin, leukopenia, bleaching of the hair and, exceptionally, agranulocytosis.

Intravenous or intramuscular injections of chloroquine, given at the usual dose of 200–300 mg base, are generally well tolerated by adult patients. However, even proportionally adjusted doses of chloroquine given parenterally to children may occasionally produce serious effects on the central nervous and cardiovascular systems. Intramuscular injections must not be given to children unless absolutely necessary and then preferably in 2 or 3 divided doses; intravenous injections are still less advisable. Hydroxychloroquine or amopyroquine seem to be safer, but experience with these 2 compounds is limited.

Acute oral poisoning by chloroquine and other 4-aminoquinolines may occur when a dose of 1.5–2 g is swallowed at once. In children half of this dose may be fatal. Symptoms of acute poisoning by oral chloroquine are headache, nausea, diarrhoea, dizziness, muscular weakness and blurred vision. The urine may contain red blood cells, albumin and casts. Emergency measures consist of removal of the swallowed drug by stomach lavage and emetics. If there are depressed vasomotor function, acute respiratory or cardiac arrest and circulatory failure, the same treatment as indicated under quinine should be given. If the secretion of urine is adequate, give 2–4 litres of fluid daily to promote renal excretion of the drug. Acidify the urine with 0.5 g of ascorbic acid given to the patient by mouth every 4 hours.

Much attention has been focused recently on the problem of the adverse effects of a high dosage of chloroquine (250–750 mg daily) for the long-term treatment of diseases of connective tissue. Damage to the eyes is the most common and most important type of toxic lesion. Such damage consists of lesions to the cornea (punctate keratitis), lens opacities or, more serious because irreversible, the deposition of chloroquine on the retina. The latter type of retinopathy appears slowly and insidiously and may not be detected until some time after administration of the drugs has stopped. It was reported mainly in persons over 50 years of age, whose cumulative dosage of chloroquine over several years of treatment was over 100 g. However, a few cases were described whose total intake of chloroquine was below this amount. It should be stressed that the dosage of chloroquine for the treatment of rheumatoid arthritis is many times higher than that used for the treatment or long-term prophylaxis of malaria.

Since the 1940s chloroquine, at the dosage of 300 mg base a week, has been adopted in many parts of the world as the best protective antimalarial regimen, except in those areas where there is *P. falciparum* resistance to this compound. The French have always advocated chloroquine prophylaxis in malarious areas during seasons of high transmission at a daily dosage of 100 mg, corresponding to 600–700 mg base a week.

Over the past 30 years the reports on the toxicity of chloroquine used as a malaria prophylactic have been remarkably few and usually connected with

gross overdosage. When the observations linking the chloroquine treatment of collagen diseases with adverse effects on vision became better known, concern about the long-term use of this compound grew and gave rise to a number of not always well founded pronouncements

Recent studies on patients with rheumatoid arthritis receiving high doses of chloroquine have concluded that the risk of developing toxic retinal chloroquine damage is small or absent even in long-term treatment, provided that the daily dosage is less than 150 mg base for 10 months in a year, i.e., that the annual intake is about 50–55 g chloroquine base The results of this study indicate that the usual dosage of chloroquine for the prevention of malaria (300 mg base a week), corresponding to about 15 g per annum, is well within the generally safe limit for at least 2–3 years Even the higher dosage—up to 700 mg base a week – may be justified in highly malarious areas, though the risk is greater The present consensus of expert opinion is that the *maximum* tolerable cumulative dosage of chloroquine is 100 g of base, corresponding to 3–6 years of continuous prophylaxis (for an adult) depending on the weekly dosage Nevertheless some reports from West Africa indicate that the haphazard use of chloroquine without medical advice is not uncommon high doses of this drug taken over years have led to cases of retinopathy in Africans, whose highly pigmented retina has a tendency to cumulate the chloroquine deposit In these circumstances, as also in some individual patients in whom factors such as hypersensitivity or the concurrent use of drugs such as some tranquillizers and antibiotics operate, some caution in the use of chloroquine and other 4-aminoquinolines for the prevention of malaria over a long period of time is justified

Biguanides proguanil (chlorguanide)

The main compounds of this series (proguanil and chlorproguanil) have been extensively investigated in a wide range of laboratory animals and although the results have varied according to the animal species the general tolerance of these drugs was found to be remarkably good This was fully confirmed by clinical and field trials in human volunteers, in whom daily doses far in excess of those normally used produced only mild gastrointestinal effects Thus at the generally accepted daily dosage of 100–200 mg proguanil or 20 mg chlorproguanil once a week side effects are extremely rare Nevertheless, some persons may experience loss of appetite, abdominal discomfort or nausea Haematuria has been described in cases of gross overdosage of proguanil

Cycloguanil - the dihydrotriazine metabolite of chlorguanide has been used in the form of the pamoic acid salt as a repository antimalarial drug in over 11 000 subjects who had up to 3 intramuscular injections of this preparation at intervals of about 3 months Local reactions at the site of the injection occurred in some 14 % of the subjects treated However, with improvement in the technique of intramuscular administration effects such as

sterile abscesses and sinus formation have been greatly reduced. Mild induration and tenderness are seen in about 10 % of subjects Side effects such as urticaria, rashes and itching have been reported in 5–10 % of cases. No significant antifolic activity or teratogenic effect has been seen

Diaminopyrimidines (pyrimethamine trimethoprim)

Toxicological studies of pyrimethamine in experimental animals have shown differences in the acute as opposed to the long-term adverse effects. They have been largely confirmed in human subjects

Accidental poisoning by pyrimethamine has been observed mainly in small children who have ingested 4–10 tablets (100 mg–250 mg) of the drug The symptoms are convulsions, loss of consciousness and collapse, frequently with a fatal outcome This alarming danger of the drug being swallowed by children has now been minimized by a new presentation in containers that cannot be easily opened by a small child

Studies on the long-term administration of pyrimethamine showed that adults tolerated doses of 100 mg a week for periods of up to 3 months

The conventional dosage of 25 mg once a week was well tolerated for 6 months or more However daily oral doses of 25 mg given for 7 weeks caused megaloblastic anaemia in a large proportion of adults, a rapid improvement followed the cessation of treatment It appears that the usual dosage of 25 mg once a week is generally safe for long periods In tropical countries, where anaemia associated with pregnancy is common, the daily administration of 10 15 mg folinic acid (racemic mixture) will forestall any significant haematological changes in women on a weekly or other regimen of pyrimethamine

It should be remembered that pyrimethamine at high doses (25–50 mg) daily for about a month is used for the treatment of acute toxoplasmosis. This regimen may cause gastrointestinal disturbances, ulceration of the mouth, loss of hair etc In patients on this regimen the need to correct any haematological toxic effects of the drug is imperative

Acute accidental poisoning by pyrimethamine (mainly in children) should be treated by gastric lavage in the first instance Convulsions are controlled by 5 10 mg diazepam given by slow intravenous or intramuscular injection Folinic acid 10 20 mg daily or sodium folate 15 mg daily should prevent or reduce the adverse effects on the haemopoietic system

Trimethoprim is generally used in combination with sulfonamides It appears that the effects of prolonged administration of trimethoprim (100 mg daily) with sulfamethoxazole are seen after about a month, or occasionally sooner The resulting depression of the bone marrow with accompanying haematological changes may occur in pregnancy or in elderly persons and should be corrected by the administration of folinic acid

Although high doses of pyrimethamine and trimethoprim have been found to produce teratogenic effects on gravid rats, there is no evidence that the conventional doses of these drugs used in the chemotherapy of malaria have an adverse effect on pregnant women and their babies.

Sulfones and sulfonamides

The main sulfones of interest in the chemotherapy of malaria are dapsone, acedapsone and the diformyl derivative of dapsone, but most of the information on the toxicity to man of this series of compounds comes from the use of dapsone. Some early studies indicated that the highest tolerated daily dose was 200 mg and that higher doses usually caused haematological effects with anaemia. Dermatitis and hepatitis occurred in patients taking 100 mg of dapsone daily. Subsequent studies showed that dapsone causes haemolytic effects in persons with G6PD deficiency when given in doses of 50 mg daily; in some such subjects 25 mg daily has also resulted in methaemoglobinaemia and haemolysis. Severe agranulocytosis has been observed after a course of several months of dapsone in 16 highly sensitive patients, 8 of whom died.

Other side effects occasionally reported are nausea, vomiting, headache, insomnia and blurred vision.

Adverse effects of short-acting sulfonamides have been described in many studies. They are mostly skin eruptions and gastrointestinal disturbances (nausea, vomiting, mild jaundice). The skin rashes can be urticarial, erythematous, maculopapular, morbilliform or purpuric and have been reported in all age groups. A severe but fortunately rare adverse effect is the Stevens–Johnson syndrome (erythema multiforme major), consisting of fever, sore throat, chest pains, arthralgia and a variety of cutaneous or mucous membrane lesions, occasionally followed by toxic epidermal necrolysis, or Lyell's syndrome, in which large flaccid blisters form. The fatality rate of this complication is about 25%. Haematological reactions in the form of granulocytopenia, agranulocytosis, aplastic anaemia and thrombocytopenic purpura have been observed. It seems that any administration of diuretics (thiazides, frusemide) before or with the sulfonamides may carry a special risk of thrombocytopenia.

Sulfonamides share common metabolic pathways and plasma-binding sites with other drugs. Thus they potentiate the effect of tolbutamide and may cause hypoglycaemia in diabetics on oral maintenance treatment. They compete with bilirubin for plasma-binding sites and, in some cases, when given in the third trimester of pregnancy, have precipitated or exacerbated kernicterus in the newborn.

The main interest of sulfonamides in the chemotherapy of malaria lies in the long-acting compounds such as sulfalene, sulfamethoxypyridazine, sulfadimethoxine and sulfadoxine, which have a plasma half-life of between 40 and 200 hours (see Table 5).

It must be admitted that, since the use of these recent drugs together with antifolic compounds is generally limited to areas where *P. falciparum* resistance to the 4-aminoquinolines is present, reports on their adverse effects are scanty It appears, however, that in recommended doses they are generally well tolerated, though gastrointestinal symptoms have been reported. There is no evidence that their use at a proper dosage is followed by haemolytic effects in G6PD-deficient individuals. They should be avoided during the first 3 months of pregnancy and should not be given to infants. If there is idiosyncrasy to the sulfonamides generally, they must not be administered. In case of any adverse effects from these compounds, administration of folinic acid at a dosage of 10-30 mg daily is indicated

Antibiotics

In view of the resistance of *P falciparum* to chloroquine and sulfonamide pyrimethamine combinations in some parts of the world, tetracycline, in association with quinine, has become an essential antiplasmodial drug However, the use of tetracycline, as of other antibiotics, entails certain adverse effects

While minor side effects of tetracyclines in the majority of patients can be disregarded, in certain sensitive individuals these drugs may cause stomatitis and intense itching of the vulva and anorectal region The suppression of normal bacterial flora makes the patient susceptible to infection with tetracycline-resistant bacteria such as staphylococci More recently, much attention has been given to antibiotic-associated colitis, which may be either pseudomembranous colitis or acute non-specific colitis The former may range in severity from mild and self-limiting to fulminating and fatal The usual initial clinical features (diarrhoea, blood and mucus in the stools, abdominal pain, fever, dehydration and a high leucocyte count) are not specifically diagnostic The symptoms have been reported after treatment with various antibiotics but more commonly clindamycin, lincomycin, tetracycline and erythromycin Recent studies show that the causative agent is *Clostridium difficile*, which produces a cytopathic toxin in the intestine. When the condition is suspected the first step is to discontinue the antibiotic and to institute rehydration and electrolyte replacement Oral vancomycin (500 mg every 6 hours for 4-6 days) appears to be effective Diphenoxylate hydrochloride with atropine and other agents that decrease intestinal motility should be avoided The value of cortisone remains to be defined. Another type of complication is excessive growth of *Proteus* and *Pseudomonas* in the urinary tract.

Apart from hypersensitivity effects (e.g. erythema multiforme), untoward reactions to antibiotics are related either to high dosage or to normal dosage in patients with renal or hepatic insufficiency. Most antibiotics rely on adequate renal function for their excretion, the exceptions being doxycycline, chloramphenicol, erythromycin, lincomycin and clindamycin, all of which are cleared

through the liver. Since penicillin and its derivatives are hardly ever given for the treatment of malaria, the adverse reactions to these compounds are not mentioned here. Tetracycline may cause an acute rise in the blood urea and creatinine levels and must not be given to patients with renal impairment Tetracyclines given in pregnancy may precipitate acute hepatotoxicity

The tetracyclines act as chelating agents and, when calcium is bound in this way in the body fluids, the yellow or brown antibiotic complex may be deposited in the teeth and bones, it affects the bone growth of the fetus and young infant and causes enamel hypoplasia

In view of the above-mentioned side-effects, tetracyclines are contraindicated during pregnancy and in children under 8 years of age

In summary, many of the unwanted effects of antibiotics in malaria chemotherapy can be avoided if their use is strictly limited to the treatment of multiresistant *P falciparum* infections

CHAPTER 7

PREVENTIVE USE OF ANTIMALARIAL DRUGS

Chemoprophylaxis and Suppression[1]

Chemoprophylaxis (or drug prophylaxis) implies that drugs are used before infection takes place or prior to its manifestation, with the aim of preventing either of these occurrences. Thus drug prophylaxis may refer to absolute prevention of infection (causal prophylaxis) or to suppression of parasitaemia and its symptoms (clinical prophylaxis).

Causal prophylaxis aims at destruction of the pre-erythrocytic forms of the parasite. The drugs employed are primary tissue schizontocides, which eliminate the infection before the merozoites are liberated into the blood stream or, in other words, before the end of the prepatent period.

Clinical prophylaxis or suppression aims at early action on erythrocytic forms when they are released by the primary tissue forms. All blood schizontocides are suppressive drugs when taken in regular small doses. When an effective suppressive is being taken there are no persisting erythrocytic parasites and thus no clinical symptoms of malaria. When administration of the suppressive drugs is continued until complete depletion of the exo-erythrocytic and erythrocytic stages of the parasite, no parasitaemia or clinical symptoms appear even after cessation of drug administration. This indicates that suppressive cure of the infection has been achieved. For *P falciparum* this would occur in about one month from the last infective bite, but for *P vivax* a much longer period would be required.

Causal prophylaxis is easily attained in falciparum malaria since the parasite in its primary tissue phase is sensitive to some of the drugs used for suppression but the other parasites are not.

Antifolic compounds such as proguanil and pyrimethamine are essentially prophylactic drugs that act on the pre-erythrocytic forms, especially of *P*

---

[1] Suppression of parasitaemia does not mean that the parasites are merely held at a low submicroscopic level during the period of drug administration. In the case of *P falciparum*, a parasite with brief exoerythrocytic development, the infection is eliminated and no subsequent parasitaemia occurs if drug administration is stopped after having been maintained for at least 4 weeks following exposure to malaria risk. In the case of parasites with persisting exoerythrocytic forms such as *P vivax* parasites may enter the peripheral blood circulation after the drug has been stopped. The interval between discontinuation of the drug and patency is determined by the particular strain of parasite, it will be short for Chesson (tropical) strains and longer for strains from temperate zones.

falciparum, present in the liver cells and evolving from the sporozoites. None of the drugs known at present acts on the sporozoites themselves during the short period between their inoculation by the mosquito and their implantation in the liver cells.

Primaquine, a compound of the 8-aminoquinoline group, also has a potent action on the pre-erythrocytic schizonts of all species of human plasmodia. However, it is not used for prophylaxis because of its possible adverse effects.[2]

All therapeutic, i.e., schizontocidal, drugs are good suppressives When taken in comparatively small doses they eliminate the parasites present in the red blood cells, or at least keep the number of plasmodia at such low levels that they provide protection from the effects of the infection. This can be achieved for prolonged periods when the compound used is appropriate and adequate doses are taken regularly

Quinine, once the common protective drug, is now seldom, if ever, used for suppression of malaria because large doses were required in some areas and its long-term administration in small doses has been associated with the occurrence of blackwater fever Chloroquine and amodiaquine are excellent suppressive drugs and have few adverse effects Various combinations of sulfones or sulfonamides with antifolic compounds are being promoted and increasingly used for the prevention of malaria It should be remembered that any prophylactic or suppressive drug may fail partially or fully in those malarious areas where there is plasmodial resistance to it

Individual drug protection

Anyone visiting or living in a malarious area can protect himself from the disease by taking appropriate drugs The term "prophylaxis" is often used in a general sense to include the preventive action of any antimalarial compound, although the specific mechanisms of action may be different in different compounds. Thus infection with *P. falciparum* ends in the pre-erythrocytic phase when proguanil or pyrimethamine is used for chemoprophylaxis, the parasites not developing to the erythrocytic stage When prophylaxis is subsequently stopped on leaving the malarious area, no parasites remain in the body to produce an attack of falciparum malaria. The pre-erythrocytic stage of *P falciparum* is more sensitive to proguanil than is the erythrocytic stage, so that successful prophylaxis can sometimes be achieved even though

[2] What amounted to continuous radical treatment was used by the United States Armed Forces in Viet Nam, chloroquine and primaquine being administered together for chemoprophylaxis With this combination primaquine acts against the tissue phase and chloroquine eliminates the erythrocytic stages of parasites If continued for a sufficient period after the last infection with *P. vivax* its use results in radical cure of infection with that species (as well as with *P falciparum*) In the presence of continued reinfection with *P vivax* the use of primaquine in combination with chloroquine offers no advantage over chloroquine alone a separate course of radical treatment could be given to eradicate vivax infections when the patient leaves the malarious area

the trophozoites show resistance to proguanil. In general neither proguanil nor pyrimethamine is active against the pre-erythrocytic stage of *P. vivax*, although pyrimethamine may occasionally be

With the drugs commonly used for chemoprophylaxis, infection with *P vivax* proceeds without interruption through the pre-erythrocytic stage, at the end of which a few trophozoites appear in the circulating blood, their presence being demonstrable by subinoculation of blood into a suitable recipient. The parasites rapidly disappear from the blood under the influence of the chemoprophylactic drug, as do their successors when they emerge from the exoerythrocytic stage in the liver from time to time while the drug is being taken However, if the drug is discontinued on leaving the malarious area and erythrocytic parasites subsequently emerge from the exoerythrocytic stage, they increase in number and an attack of vivax malaria results

As *P. falciparum* does not have a persistent exoerythrocytic stage in the liver, the rapid destruction of erythrocytic trophozoites as they emerge from the liver means that infection with drug-sensitive strains of this species is eliminated by the chemoprophylactic drug When the drug is discontinued, 4 weeks after the last possible exposure to infection, no subsequent attacks of falciparum malaria should occur

Infections with *P ovale* and *P malariae* respond in a similar way to *P vivax* when exposed to the drugs used for chemoprophylaxis

The drugs commonly used for personal prophylaxis together with the appropriate dosage and the frequency of administration are shown in Table 9 All these drugs are effective from the first day of administration (in areas where the parasites are sensitive to the drugs) but, for reasons explained below, it is advisable that regular drug-taking should start 1–2 weeks before entering the malarious area The drug should be taken for at least 1 month after leaving the malarious country to ensure that infection with *P falciparum* is eliminated

Proguanil is remarkably free from toxic effects. When used for chemoprophylaxis it acts on the pre-erythrocytic stage of *P falciparum* and on the trophozoites of all 4 species Resistance of *P falciparum* to proguanil has been reported from many areas and resistance of *P vivax* has also been reported (see Chapter 5)

Pyrimethamine is free from toxic effects at the doses recommended for the prophylaxis of malaria. It has much the same activity as proguanil and is also active against the pre-erythrocytic stage of some strains of *P vivax*. As it has a sweetish taste and accidental poisoning of children has been reported on a number of occasions, the tablets should not be accessible to children.

Amodiaquine and chloroquine are usually well tolerated in the doses recommended for the prophylaxis of malaria. After prolonged administration of amodiaquine pigmentation of the palate, alae nasi and nail beds may be seen. Difficulties in visual accommodation, which may occur with therapeutic doses of chloroquine, are not normally experienced with prophylactic doses.

When high doses of chloroquine are used continuously for a number of years the compound may accumulate in the retina, producing a loss of visual acuity. However, with the dosage (300 mg a week) of chloroquine required for the prevention of malaria this adverse effect is not known to occur unless the drug is taken continuously for more than 6 years, when the cumulative amount exceeds 1·00 g. Even at this dosage level, chloroquine retinopathy appears to be uncommon.

It has been suggested that, in areas where there is a high level of transmission the amount of drug (e.g., proguanil, chloroquine, amodiaquine) taken for chemoprophylaxis may be doubled.

If the weekly intake of chloroquine base is of the order of 600–700 mg the overall duration of administration should not exceed 3–$3\frac{1}{2}$ years. When the strains of parasite present in the area are normally susceptible to the drug being used for prophylaxis any apparent failure of the drug to protect from malaria is more likely to be due to non-compliance with the regular regimen rather than to an inadequate dose being used.

Individual drug protection in areas where the presence of drug-resistant strains of plasmodia has been confirmed presents some difficulty when it comes to the choice of an appropriate compound. This applies particularly to infections with *P. falciparum*. If a high degree of resistance to proguanil or pyrimethamine is present and the parasites respond adequately to the 4-aminoquinolines, chloroquine or amodiaquine is the obvious drug for chemoprophylaxis. In countries or areas where strains of *P. falciparum* are highly resistant to proguanil, pyrimethamine and the 4-aminoquinolines, there is no entirely satisfactory drug or drug combination available at present, although some newer compounds offer considerable promise of being effective and safe. For the time being each case must be given individual attention, in the light of the country or area involved and the probability of the person or persons being infected.

If nonimmune individuals such as tourists are going to spend only a limited amount of time in an area where there are strains of *P. falciparum* resistant to the 4-aminoquinolines, a choice should be made from one of the drugs listed in Table 9. It is advisable to inform the traveller of the possibility of infection occurring in spite of treatment, so that he will seek medical advice should this occur.

If the time to be spent in a resistant area is longer or if infection with *P. falciparum* strains resistant to the 4-aminoquinolines is likely to occur, certain combinations of drugs may be used. A proprietary combination (Maloprim) of pyrimethamine (12.5 mg) and dapsone (100 mg) taken once a week has been widely used with satisfactory results. A further combination consisted of 200 mg proguanil and 25 mg dapsone taken daily. This proved to be very effective in military contingents in south-east Asia, but adverse effects on the blood (agranulocytosis) were seen in some individuals who took the 2 drugs for a period of over 1 year (Black, 1973). The same effects

occurred in subjects given a weekly dose of chloroquine, primaquine and dapsone. The incidence of agranulocytosis was of the order of between 0.1 and 0.5 per 1000 per year in people taking proguanil and dapsone, so the chances of one individual suffering from this condition are very small. However, agranulocytosis can have serious consequences and for this reason the combination should be used without exceeding the usual dosage.

There is also increasing evidence of the value as a preventive drug of a proprietary combination of sulfadoxine (500 mg) with pyrimethamine (25 mg) given at the adult dosage of 1 tablet once a week. Since there are few data on the long-term use of this drug combination it may be preferable to use it only for a limited time (4–6 months) until further information becomes available (Table 10).

A few practical points related to the individual protection of travellers or visitors to malarious areas should be mentioned.

A wide variety of people travel to malarious countries and remain there for varying lengths of time. They comprise tourists (individuals and organized

TABLE 10 COMBINATIONS OF ANTIMALARIAL COMPOUNDS USED FOR TREATMENT OR FOR INDIVIDUAL AND COLLECTIVE PROTECTION

Non proprietary name	Formulation	Some proprietary names	Dose for prevention (adult)[1]	Dose for treatment (adult)	Remarks
Pyrimethamine and chloroquine sulfate	25 mg + 150 mg (base)	Daraclor	1 or 2 tablets once a week. Children under 6 years of age ½ a tablet	Not generally used	Mainly for single dose treatment (presumptive treatment) of cases suspected of malaria infection prior to confirmation of the diagnosis. Used in malaria eradication programmes
Amodiaquine and primaquine	150 mg (base) + 15 mg (base)	Camoprim	2 tablets once a week for limited periods	2 tablets the first day, 1 tablet on the next 2 days, then 2 tablets once a week for 4–6 weeks	Mainly for limited mass drug administration programmes. To be used with caution in dark skinned individuals. Also available as Camoprim Infatab at half the dosage of amodiaquine. For limited paediatric use
Pyrimethamine and dapsone	12.5 mg + 100 mg	Maloprim	2 tablets before exposure, then 1 tablet once a week	Not generally used	To be used for individual protection. Not for children or pregnant women. May be used with caution in areas with resistance of *P. falciparum* to other drugs
Chloroquine and chlorproguanil	150 mg + 20 mg	Lapaquin	1 tablet a week	Not normally used	Mainly for individual prevention of malaria but occasionally used for limited mass drug administration
Pyrimethamine and sulfadoxine	25 mg + 500 mg	Fansidar, Falcidar	1 tablet a week or 2 tablets every 2 weeks	2–3 tablets as a single dose	For treatment of falciparum malaria resistant to chloroquine and other drugs. Liquid formulation available for parenteral use[2]
Pyrimethamine and sulfalene	25 mg + 500 mg	Metakelfin	As above	As above	Dosage of tablets as above. No injectable formulation available

[1] For limited periods (3–6 months) in areas where resistance to other drugs has been confirmed.

[2] Each ampoule of 2.5 ml contains 25 mg of pyrimethamine and 500 mg of sulfadoxine.

parties), professional, technical and business people, university staff and students, schoolchildren on holiday travel, missionaries, armed forces personnel, tradesmen and others, and some have their families with them. In certain cases, too, local key personnel require the protection. All require briefing in individual malaria chemoprophylaxis and other measures to protect themselves from malaria The briefing must be done carefully so that the measures are fully understood, and stress should be laid on the possible effects of failure to take precautions in order to promote the necessary motivation The subject of what measures to take for individual protection from malaria should be brought up when travellers are receiving their immunizations Information on areas with a risk of malaria is helpful for such briefing [3]

The advice on the best protective drug varies according to the intensity of malaria transmission, the degree of exposure to infection and the type of malaria prevalent in the area concerned It is difficult, if not impossible, to lay down a single rule to fit every situation

An appropriate drug should be prescribed so that the traveller can obtain sufficient supplies to cover the period of his visit and at least 1 month after leaving the malarious areas Some method of supervision is necessary to ensure that the tablets are taken For the individual this may take the form of an entry in a diary at the time the tablets are actually taken Parents should supervise their children and tour conductors the tourists in their parties In the armed services stricter supervision is possible

The drugs, especially chloroquine, are best tolerated when taken after a meal to reduce the occasional occurrence of nausea It is an advantage to start the drug regimen a few days before departure since the traveller can begin to establish a routine moreover, the existence of idiosyncrasy to any drug will be discovered while it is still possible to change the active compound Whatever the drug and the regimen selected, it must be followed with unfailing regularity to be fully effective One single omission, especially of a weekly dose, interrupts the protective effect In view of this the regular daily intake of a drug such as proguanil is a distinct advantage

In addition to the taking of a chemoprophylactic drug, there are many other measures that reduce the chance of malaria infection The wearing of long sleeves and trousers after dusk decreases the chances of being bitten by anopheline mosquitos, and the application of mosquito repellents to the exposed skin at night has the same effect Other measures include sleeping under a mosquito net, spraying the room with a pyrethrum knockdown aerosol, protecting living quarters by mosquito screens, avoiding villages at night and siting camps for the nonimmune 1–2 kilometres away from villages or other local habitations

[3] The latest information together with a map is available in *Weekly Epidemiological Record*, No 22, 1979 (see Fig 1 for map)

The traveller should also be warned that vivax malaria may develop after he has stopped taking the drug a month after his return. He should inform his physician of his possible exposure to infection if he becomes ill after his return.

Routine radical treatment for all travellers returning from malarious countries is impracticable and often unnecessary It could well be given to certain categories of people who have probably been infected with malaria because of the nature of their work, for example field workers, anthropologists, missionaries and personnel of the armed services, but for others it is a matter of individual assessment A passenger arriving from a malarious area may sometimes be required to take a prescribed course of treatment as part of maintenance phase activities in a country where malaria eradication has been achieved

Finally, it is important to warn pregnant women that an attack of malaria is a threat to pregnancy and assure them that such drugs as chloroquine and proguanil will have no untoward effect on the fetus However, combinations of pyrimethamine with sulfonamides are not recommended during the first trimester of pregnancy

Drug Administration in Malaria Control Programmes

Collective drug protection

The general protection of groups of people residing in malarious areas and of populations living there permanently can be achieved temporarily by collective chemotherapeutic measures

This method has been used with success in army units, organized labour forces or similar communities The rapid excretion of all existing drugs means that they must be administered frequently and regularly This demands organization, efficient distribution and, above all, persuasion

In programmes drugs are used to give protection to particular categories of people In some countries, however, a more general distribution is made in an attempt to protect the whole population This may be necessary as the immediate first step in an epidemic of malaria, to be followed up by more lasting control measures

It is obvious that collective drug protection differs only in degree from the mass drug administration described in the next section

In countries where, for any reason, a malaria eradication programme cannot be undertaken the health service may carry out drug distribution as one of the services provided in the rural areas, the aim being to prevent or reduce the effects of malaria by the use of schizontocidal drugs. Although transmission of the infection cannot be interrupted by this measure alone it has definite beneficial effects, since it not only protects individuals but may also gradually reduce the reservoir of infection by decreasing transmission by the mosquito

In carrying out such a programme special attention should be paid to a number of factors.

(1) the value of the method as a public health measure,
(2) the question whether the programme should attempt to cover the whole population or only specific categories of people,
(3) the possibility of undesirable long-term side effects on the community,
(4) the selection of the appropriate drug and dosage, and
(5) the method of drug distribution and the timing, regularity, frequency and supervision of its management and effects

With regard to (1), there is no doubt that collective drug distribution is of immediate benefit to the indigenous population living in a malarious area It has been shown in Africa that regular drug distribution decreases the total amount of sickness from all causes, to some degree reduces absenteeism in schools, and may be followed by modest but definite gains in weight and an increase in blood haemoglobin

With regard to (2), it is obvious that collective drug distribution must be adapted to the epidemiological conditions of the area In areas of moderate endemicity and seasonal transmission all groups of the population benefit from drug distribution (adjusted to the start of the transmission period), while in highly endemic areas the long-term protection of the younger more vulnerable age groups is preferable It is impossible to administer any drug to the entire population or ever to a particular category of people with absolute regularity However, a less than total coverage may, depending on the level of transmission, have an appreciable effect on the amount of malaria

With regard to (3), the possible undesirable long-term effects of distributing an antimalarial drug regularly should be considered from two angles the toxic action of the drug and its possible interference in highly endemic areas with acquired tolerance to the infection As far as the first point is concerned, it seems that, with the exception of mepacrine and some 8-aminoquinolines, the harmful effects of most of the well-known drugs are very few, particularly when assessed in the light of the benefits that the drugs confer Definite information about the second point is lacking, probably because in all field trials absolute regularity of drug distribution has never been achieved and reinfection, even of short duration, has been sufficient to maintain a degree of immunity

With regard to (4), the selection of an appropriate drug for collective protection, the general principles outlined in this manual are valid A good schizontocide, if given at an adequate dosage, acts on all the 4 species of human plasmodia in their asexual stage in the erythrocyte cycle and has a slow effect by attrition on the gametocyte reservoir For this purpose the 4-aminoquinolines are unsurpassed and there is little to choose between amodiaquine and chloroquine Proguanil and pyrimethamine have a causal prophylactic effect and also a direct sporontocidal action on the gametocyte reservoir Their large-scale use is not justified where there is continuous and high-level transmission, because of the probability of the selection of resistant

strains in a population already infected. If these drugs are given, periodic assessment of their effect must be made. Their use in a combined form with a 4-aminoquinoline is much less open to objection. In areas where chloroquine-resistant strains of *P. falciparum* occur the protection to be expected from the 4-aminoquinolines will be less than elsewhere. Furthermore, it is to be expected that their use would exert selective pressure in favour of the resistant strains. The use of alternative drug combinations in restricted areas might be considered, but the widescale use of combinations containing sulfonamides or sulfones entails the risk of inducing sulfonamide resistance in such important pathogenic bacteria as the meningococci

With regard to (5), the frequency of administration is related not only to the dosage of the drug but also to the convenience of its distribution. In general once-weekly administration is the most appropriate, though fortnightly distribution may be adequate. The frequency of drug administration depends on many local conditions and the level of transmission, but a reasonably strict observance of weekly or fortnightly routines is not too difficult. In schools this regimen is certainly the most suitable and minimizes the effect of a default or two in the weekly drug distribution. It is obvious that in areas of high endemicity the risk of reinfection is greater when treatment is spread more widely. Breaks in this drug distribution in schools owing to holidays are unavoidable.

There is little doubt that in collective drug protection two groups of the population must be given the highest priority: pregnant and nursing women and infants and children. The distribution of drugs to these two groups is not difficult through the normal health service and schools, but a proportion of women and children will always be missed since total drug coverage is almost impossible to achieve in rural areas. In highly malarious areas regular once-weekly or once-fortnightly administration of drugs to small children attending clinics for the under-fives is of special value since malaria can be severe and often fatal in children of this age group.

Drug protection from malaria should be the responsibility of the national health service and the cost must be met mainly by the government, though bilateral or multilateral aid agencies may provide substantial assistance in the organization and expenditure involved.

Table 11 shows the dosage of the main drugs that can be given for collective drug protection in relatively small groups with little immunity or in semi-immune communities living in an endemic area.

At the appropriate dosage none of these drugs taken for general protection has any serious side effects. Proguanil and pyrimethamine have a wider margin of acceptability than the 4-aminoquinolines.

Epidemics of malaria

Epidemics of malaria require special attention. For the control of epidemic malaria in rural communities the above doses are inadequate and the following dosage of fully active schizontocidal drugs is recommended:

	Immediate single adult dose	*Follow-up adult dose*
Chloroquine	600 mg base	300 mg base once a week
Amodiaquine	600 mg base	400 mg base once a week

Special responsibility lies with the distributors of the drug, they must ensure that the drug is really swallowed and not vomited and that the whole population takes the drug. Drug distribution should continue for 1 month after the confirmed end of the epidemic The possibility of relapsing vivax and recrudescent quartan malaria some weeks or months after the cessation of the drug distribution should be taken into account

TABLE 11 DOSAGE OF DRUGS FOR COLLECTIVE PROTECTION

Drug	Groups with little immunity (adult dosage)	Semi immune communities in endemic malarious areas (adult dosage)
Proguanil	100 200 mg daily	300 mg once a week[1]
Pyrimethamine	25 50 mg once a week	25 mg once a week
Chloroquine	300 600 mg base once a week	150 300 mg base once a week
Amodiaquine	400 mg base once a week	200 400 mg base once a week

[1] There should be regular monitoring of the response of malaria parasites to these compounds and replacement by alternative drugs if resistance occurs

Various drug combinations (chloroquine with pyrimethamine, chloroquine with chlorproguanil, amodiaquine with primaquine, pyrimethamine with dapsone) have been used for the protection of relatively small groups, with results ranging from good to disappointing This is generally related to the regularity and completeness of the method of drug administration and reflects the degree of acceptance of the drugs and the motivation of the population

The repository drugs have not yet found a well-defined place in malaria control programmes but could be used effectively in special situations.

The use of drugs in malaria control and eradication programmes

Before describing ways of using antimalarial drugs in malaria eradication programmes it should be stressed that investigation of the response of local strains of parasites to the drugs proposed for use ought to be part of the preparatory phase and the response should be continually monitored. In this way the presence or appearance of drug-resistant strains can be detected early and appropriate alternative drugs be used.[4]

The three main types of treatment with drugs in malaria eradication programmes are presumptive treatment, mass drug administration and radical treatment

[4] WHO Technical Report Series No 529, 1973 *Chemotherapy of malaria and resistance to antimalarials* Report of a WHO Scientific Group

Presumptive treatment

This is the treatment given to a person presumed to have or suspected of having malaria It consists of a single dose of a 4-aminoquinoline together with either a gametocytocide or a sporontocide The objective of presumptive treatment is to relieve symptoms and prevent transmission until the diagnosis is confirmed and radical treatment can begin

In practice presumptive treatment consists of administration of 450–600 mg chloroquine or amodiaquine base with the addition of either 30-45 mg primaquine or 25–50 mg pyrimethamine (adult doses) At the same time a blood film is taken and if it is positive a course of radical treatment is given. The doses for children should be reduced proportionately

In areas where chloroquine-resistant strains of *P falciparum* are widespread an alternative schizontocide should be given This may be 50 mg pyrimethamine together with 1 g sulfadoxine or 2 g sulfalene For presumptive treatment, primaquine (30–45 mg once weekly) may be considered as an addition during the transmission period since pyrimethamine will not affect the gametocytes when pyrimethamine resistance is widespread

The single-dose treatment of malaria given in clinics or outpatient departments differs from presumptive treatment in that it does not contain a gametocytocide or a sporontocide It is often used in areas of hyperendemic malaria where there is no malaria eradication programme In such areas the appropriate schizontocidal component of drugs used for presumptive treatment should be administered for single-dose treatment

Mass drug administration

Mass drug administration is the distribution of a specified drug to every individual in a given population This may mean the total population of a malarious area or particular groups such as children, pregnant women, or members of a work force within the total population

In malaria eradication programmes mass drug administration may be used in localized areas (1) where small foci continue to persist after transmission has been interrupted elsewhere, (2) for a focal outbreak in the consolidation or maintenance phase in addition to insecticide spraying and other measures, (3) in situations where there is population movement and people congregate from various parts of the country Mass drug administration may also be used as a supplementary attack phase measure when residual insecticide spraying does not fully interrupt transmission, but it is not a substitute for proper spraying.

Numerous difficulties attend the use of mass drug administration, it is therefore not a procedure that should be adopted without very careful consideration Operational problems include staff difficulties in administering the drug, the identification of individuals for the purpose of record-keeping, population attitudes and beliefs, and the highly organized and costly system of distribution required to carry out the procedure effectively Technical

problems include those arising from the frequency of drug administration necessary with the drugs at present available, the difficulty of achieving the total coverage required if the procedure is to be effective, the possible occurrence of side effects, which may be real or imaginary, and the emergence of drug-resistant strains of parasites [5]

Mass drug administration can be achieved either by direct supervised distribution of tablets or by incorporation of the drug into common salt used for the normal daily preparation of food The latter form of indirect drug distribution, introduced in Brazil in the 1950s, is often referred to as Pinotti's method [6]

A number of difficulties have been met in using Pinotti's method in the field A community may draw its salt from a wide variety of sources, thus there may be considerable problems in trying to channel the supply of salt through a single point where the antimalarial compound is added It is difficult to ensure the even distribution of the active drug through salt in bulk, and in damp conditions it may concentrate or leach out into one part of the container The individual consumption of salt differs considerably, hence the range of dosage with the antimalarial varies, some people consume little or no salt and therefore escape the action of the drug Infants particularly consume little or none and they are therefore the group most at risk These difficulties have bedevilled the application of what at first sight seemed to be a simple and effective method In consequence, medicated salt distribution is likely to be of value only in very special circumstances It proved to be very successful in Guyana, Iran and Suriname, and it may be applicable in other areas where a single source of salt can be identified and controlled The procedure involves mixing a chloroquine or amodiaquine salt concentrate locally with the salt in bulk This is done by machinery (e g with a concrete mixer) and entails limited capital expenditure Nevertheless, the whole process demands a fair degree of management, of which only governments or large industrial concerns are capable

The antimalarial compounds suitable for use with medicated salt are chloroquine and amodiaquine When pyrimethamine was used for this purpose in the early field trials, rapid development of resistance to the drug invariably followed Chloroquine has been used most commonly for medicated salt distribution, though amodiaquine base, which is less bitter than chloroquine, was employed with success in Suriname

[5] WHO Technical Report Series No 375, 1967 *Chemotherapy of malaria* Report of a WHO Scientific Group

[6] See PAULINI E (1960) Guide-lines for the use of medicated salt (Pinotti's method) in malaria eradication programmes (unpublished document WHO/MEM/1)

The general requirements for this method are as follows

(1) The salt supply of the population must be such as to ensure that only medicated salt is consumed This may require legislative action and much public health education

(2) The salt intake must be regular and well known so that the concentration of the compound in relation to the average daily consumption of the inhabitants can be calculated

(3) The final concentration of the drug must be adjusted so that the weekly dosage of chloroquine or amodiaquine base is 300 400 mg

(4) The concentration of chloroquine in the salt must not exceed 4g/kg as beyond this limit the salt becomes bitter

(5) The mixing and bagging must be such as to prevent irregular drug concentration and leaching out on storage

(6) A regular follow-up of results is necessary to detect any technical, operational or human difficulties that may prevent consumption of the appropriate dosage, since the application of this ingenious method is more difficult than might be expected

In all mass drug administration programmes the response of the parasites to the drugs must be continually monitored to ensure early detection of drug resistance Possible adverse reactions to the drugs in the various age groups should also be borne in mind

Radical treatment

Radical treatment of vivax malaria has been discussed in detail in Chapter 6 A number of factors may necessitate modification of the amount of primaquine usually given for radical cure of vivax malaria In addition, in malaria eradication programmes it is usual at the appropriate stage to give radical treatment in all cases of malaria, including those caused by *P falciparum*, since there may be latent infection with *P vivax* as well as overt falciparum parasitaemia and since primaquine destroys any surviving gametocytes of *P falciparum*

In many programmes the course of radical treatment lasts for only 5 days, during each of which 15 mg primaquine are administered under supervision, 1 5 g chloroquine base also being administered in the first 3 days The reason for the shortness of the course lies in the necessity to supervise the treatment, which is operationally possible for 5 days but hardly ever for 14 days This shortened course does not always succeed in eradicating *P vivax*, but relapses should be picked up by surveillance procedures, as by active or passive case detection and the follow-up of treated cases

In the south-west Pacific larger amounts of primaquine than usual are required for the radical cure of some strains of *P vivax*, and daily doses of 22.5 mg primaquine for 14 days are commonly used Even with this regimen

relapses may occur This regimen presents operational difficulties if it has to be used as a routine measure in an eradication programme

Mass radical treatment has been used in some areas, but the areas must be very localized with small numbers of people involved if the operation is to be carried out effectively Obviously this procedure would be a waste of time if transmission was still going on, thus when it is used for small residual foci it must be combined with effective residual spraying or carried out during that part of the year when there is no transmission Because of the length of the course of treatment, any movement of people adds to the difficulty of supervising this form of drug distribution

Prevention of Malaria Accidentally Induced by Blood Transfusion

The subject of accidental transfusion malaria has recently assumed some importance, a large number of such cases having been described and analysed Various methods of selection of donors in non-malarious areas and the possible use of immunological tests in special circumstances have been discussed The striking growth in international travel in the past decade has led to the exposure of many potential blood donors to malaria, in consequence, they may be able to transmit malaria when their blood is used for transfusion Donors are often excluded on the basis of a specified length of time since their exposure to malaria infection If this results in the exclusion of too large a proportion of the donor panel the use of radical treatment for those suspected of malaria could be considered, possibly in association with serological tests

In malarious areas nearly all donors are likely to transmit malaria when they give blood for transfusion, so that routine employment of antimalarial drugs is necessary to prevent accidentally induced infection Transfusions may be given either from *ad hoc* donors in an emergency or from a well organized panel of donors In the first case the best policy is to treat the recipient in the second it may be possible to institute routine chemoprophylaxis for all the donors on the panel

If well supervised routine chemoprophylaxis of the donor panel in a malarious area is not possible, the recipients should receive treatment to destroy any parasites they may receive with the transfused blood Where parasites are normally sensitive to the 4-aminoquinolines a standard course of chloroquine (1 5 g in 3 days for an adult) eliminates any infection acquired in this way Where drug-resistant strains are present appropriate alternative treatment should be used (see pp 131 132) The course can be started the day before a planned transfusion or at the time of transfusion Exoerythrocytic parasites do not occur in these circumstances and the use of primaquine is not indicated A combined approach may be possible using chemoprophylaxis for the donor panel and prophylactic treatment for the recipients

Cost of Mass Drug Administration

The cost of drugs alone can be relatively easily calculated, although the price of the same compound may vary from country to country because of differences in the suppliers, in transport and in customs duties However, the total cost of a mass drug distribution scheme is difficult to estimate since it must include operational and evaluation expenses In a project in Senegal that aims at protecting infants and children up to the age of 14 years the approximate cost in 1972 was US $0 16 per child per year exclusive of drug administration, the cost of which was estimated at US $0 04 In this project the drug distribution was carried out through the agricultural cooperatives

The type and frequency of mass drug administration vary so much from country to country that the cost in one cannot be used to estimate the cost in another The reported costs from Africa varied in 1973 between US $0 20 and 0 80 per person per year [7]

Another estimate prepared in 1973 indicated that in tropical Africa, for a population of 1 million of whom 75 % live in rural areas, the annual cost of drug protection by chloroquine through once-weekly chemoprophylaxis for children and pregnant women and single-dose treatment of malaria attacks in the rest of the population would be about US $185 000 or approximately US $0 185 per person per year [8] This includes the probable expense of drug distribution

However, the latest estimates, prepared for the Thirty-first World Health Assembly (1978),[9] indicate that single-dose treatment of fever cases (most of them presumably caused by malaria) under tropical African conditions using chloroquine tablets of 100 mg base, at an average dose of 10 mg/kg body weight, for the protection of one million inhabitants would require 4 500 000 tablets At the price of US $10 per 1 000 tablets the total cost of the drug (excluding the cost of its distribution) would amount to US $45 000, the cost per protected inhabitant thus averaging US $0 05 Because the costs of distribution and evaluation of the results of such a programme vary tremendously from one country to another, since they depend on wages and salaries, transport, and other incidental expenses, the above estimates are only of indicative value, though they may help health administrators to plan the intended antimalaria programmes

In conclusion, it seems that in most situations it is theoretically possible to prevent malaria by the use of an appropriate drug or combination of drugs

[7] WHO Technical Report Series, No 529 1973 *Chemotherapy of malaria and resistance to antimalarials* Report of a WHO Scientific Group

[8] WHO Technical Report Series, No 548, 1974 Sixteenth report of the WHO Expert Committee on Malaria

[9] Malaria control strategy Report by the Director-General to the Thirty-first World Health Assembly (unpublished WHO document A31 19)

Even when the element of human fallibility is reduced to a minimum, as in conditions of strict military discipline, cases of malaria still occur because absolute regularity of administration has not been achieved; individuals occasionally omit to take the prescribed doses, particularly when the routine is disturbed. It cannot therefore be expected that populations or even particular categories within populations will achieve absolute regularity and it would be impossible to provide adequate supervision to ensure that they did. For these reasons too much must not be expected from mass drug administration, especially as malaria attacks can occur even in responsible people for whom individual chemoprophylaxis has been prescribed.

ANNEXES

ANNEX 1

GLOSSARY OF TERMS AND DEFINITIONS[1]

This glossary defines some of the technical terms used in the text of this manual but not defined therein. It is restricted to terms directly connected with malaria chemotherapy. The definitions are designed solely for use with this manual and are not necessarily valid for any other purpose.

adverse effects of drugs

A term comprising all the unwanted effects produced in human subjects by an intake of drug. These effects may be loosely classified according to Rosenheim (1958)[2] as follows: (*a*) *toxic effects* are due to overdosage either by a single large dose of the drug or by cumulation; (*b*) *side effects* are therapeutically undesirable but unavoidable consequences of taking a drug (e.g. nausea and vomiting after chloroquine taken on an empty stomach, or fall of blood pressure after an intravenous injection of quinine); (*c*) *secondary effects* are those which arise indirectly as a result of an action of a drug (e.g. moniliasis in patients given prolonged treatment with a tetracycline); (*d*) *intolerance* is a lowered threshold to a normal physiological action of a drug (e.g. giddiness, deafness, blurred vision caused in some patients after a normal dose of quinine); (*e*) *idiosyncrasy* is a qualitatively abnormal reaction to a drug (e.g. haemolysis in some patients after the administration of primaquine); (*f*) *hypersensitivity* or *allergic reaction* is due to an abnormal immune response after previous sensitization by a drug (e.g. penicillin allergy).

analogue

In chemical terminology, one of a group of substances having similar properties or showing some common characteristics.

antifolic drugs (or *antifolates*)

Compounds that inhibit dihydrofolate reductase (an enzyme involved in the reduction of dihydrofolate to tetrahydrofolate) and in consequence arrest the early formation of nucleic acid involved in the growth of malaria parasites. This term is often applied to diaminopyrimidines, biguanides and related compounds.

[1] This glossary is based in part, on *Terminology of malaria and of malaria eradication. Report of a drafting committee*. Geneva, World Health Organization, 1963, and many definitions relevant to clinical pharmacology are according to Laurence, D. R., *Clinical pharmacology*, 4th ed. London and New York, Churchill Livingstone, 1973.

[2] Rosenheim, M. L. (1958) Introduction with a note on terminology. In Rosenheim, M. L. & Moulton, R. ed. *Sensitivity reactions to drugs. A symposium organised by the Council for International Organisations of Medical Sciences*, Oxford, Blackwell, pp. 1–5.

attack — A period of acute (overt) illness, consisting of a single or of several febrile periods often combined with other symptoms The first attack following the incubation period is often called the primary attack"

bioavailability — The rate and extent of absorption of a drug from a dosage form as determined by its concentration/time curve in the systemic circulation or by its excretion in urine

blackwater fever — A group of symptoms dominated by acute intravascular haemolysis with haemoglobinuria, often followed by renal failure usually associated with fever and generally due to infection with falciparum malaria

brood — Erythrocytic forms of plasmodia belonging to the same cyclical generation and at about the same stage of development

carrier A person harbouring malaria parasites with or without clinical evidence of infection

chemoprophylaxis Protection from or prevention of disease by chemotherapeutic means

clinical trial An evaluation of the action or actions of a drug on man The response can be measured in three ways (*a*) in a graded response the measured effect is related to the dose (*b*) in an all or none response the effect of a drug is judged by the presence or absence of a selected reaction (*c*) in a direct response the dose of the drug is increased until a desired effect is obtained

clone A population of genetically identical organisms derived from a single cell by asexual reproduction In malaria parasites clones are usually derived from erythrocytic forms by means of a dilution technique and *in vitro* culture

compliance Strict adherence by the patient to the prescriber's instructions regarding the method dosage and pattern of drug administration

congener In chemical terminology one of a group allied in origin i e all belonging to the same chemical group and derived from the same parent compound thus the 4 aminoquinolines are congeneric with or congeners of one another

culture A growth of malaria parasites (usually erythrocytic forms) propagated and maintained *in vitro* Such cultures are used for various experimental purposes such as evaluation of the sensitivity of the given isolate to specific drugs

cure clinical Relief of symptoms of malaria without complete elimination of the infection

cure radical Complete elimination of malaria parasites from the body so that relapses cannot occur

cure suppressive Complete elimination of malaria parasites from the body by means of continuous suppressive treatment

dosage — Amount of a drug to be given for the treatment of a particular condition usually graded in accordance with the age and weight of the recipient Dosage schedule refers to the amount of the drug and to the interval between the appropriate doses

dose	Prescribed amount of a drug to be taken at one time or within a given period The amounts of antimalarial drugs should be expressed in metric units such as milligrams (or decimal fractions of a gram) or appropriate units of volume (e g ml) in preference to any measures formerly common (e g grain)
dose adult	Amount of a drug to be given to an adult person of average weight
dose loading	Initial dose of a drug higher than that subsequently used, given with the object of rapidly providing an effective drug concentration in the blood Also known as primary dose
dose single	Quantity of a drug prescribed to be taken on a single occasion and intended to produce an effect without further medication
drug	1 A substance or mixture of substances intended to be used in the prevention mitigation or treatment of disease or of an abnormal physical state or the symptoms thereof in human beings or animals 2 Any substance or product that is used or intended to be used to modify or explore physiological systems or pathological states for the benefit of the recipient [1]
drug causal prophylactic	See under *prophylaxis causal*
drug repository	A sparingly soluble drug or drug preparation which on being injected intramuscularly or subcutaneously forms a local depot from which the active principle is gradually released into the circulation Also known as depot preparation
drug association	The simultaneous administration of two or more drugs either in separate or in compound preparations
drug failure	Absence or insufficiency of drug action after the administration of a normally effective dose It is important to discriminate between such causes of drug failure as deficient absorption unusual rate of degradation or excretion of the drug and resistance of the parasite
drug formulation	The presentation of the final pharmaceutical product, e g tablet elixir injection also the composition of the relevant formulation including the characteristics of the raw materials and the operations required to process them
drug interaction	Alteration of the pharmacological action or disposition of one drug when given together with another drug
drug monitoring	As applied to the problem of adverse effects of drugs, any procedure designed to provide systematic information on a likely causal relationship between the administration of medicinal or other substances and their abnormal effects on the relevant population The main systems of drug monitoring are (*a*) individual reporting by professionals involved in medical care of the population, (*b*) monitoring by special reference centres, or organized surveys

[1] WHO Technical Report Series, No 341, 1966, p 7

drug receptors — A convenient term to describe the constituents of a cell with which drugs react. Receptors are thought to be of molecular size and form part of the lipoprotein structure of the cell membrane. The characteristic property of receptors, like that of enzymes, is their specificity.

drug tolerance — A condition requiring that the dose of the drug be increased to obtain an effect previously achieved with a smaller dose. This term refers to the patient and is not synonymous with the development of specific drug resistance by the pathogen.

drug utilization — The marketing, distribution, prescription, and use of drugs in a society, with special reference to the resulting medical, social, and economic consequences

extender effect — Delayed degradation or excretion of a drug (or of its active metabolite) brought about by the concurrent administration of another substance.

fever — Abnormal increase of body temperature. It may be classified as (*a*) continuous, (*b*) remittent (characterized by decreases but without a return to normal temperature), or (*c*) intermittent (interrupted by periods of normal temperature).

formulae of organic compounds — Four types of formula are used for describing an organic compound: (*a*) the *empirical formula* indicating the proportions in which the constituent atoms are present in the molecule; (*b*) the *molecular formula* indicating the actual numbers of the constituent atoms in the molecule; (*c*) the *constitutional formula* indicating the grouping of the atoms in the molecule, and (*e*) the *graphical formula* indicating the position of every atom and linkage in the molecule.

gametocyte — Parent cell of a gamete. In malaria parasites, the female gametocytes (macrogametocytes) and male gametocytes (microgametocytes) develop in the red blood cell. Russian-speaking authors often refer to these forms as "gamonts".

gametocytocide — A drug that destroys the sexual forms of malaria parasites. This term commonly refers to compounds that act selectively on gametocytes of *Plasmodium falciparum* (crescents) since these are not immediately affected by the usual blood schizontocides that destroy both the asexual and sexual forms of *P. vivax*, *P. malariae* and *P. ovale*

glucose-6-phosphate-dehydrogenase (G6PD) deficiency — A genetic deficiency of an enzyme normally present in the red blood cells and involved in the metabolism of glucose by the erythrocytes. The defect of this enzyme is linked to the X chromosome and fully expressed in male hemizygotes but variably manifest in female heterozygotes.
There are two types of G6PD deficiency: type A is seen mainly in Negroes; type B, only in Caucasians of Mediterranean stock. Under the influence of certain drugs the G6PD-deficient cells undergo a degree of haemolysis resulting in haemoglobinaemia and haemoglobinuria. The drugs with such action include quinine, primaquine, sulfones, many sulfonamides, chloramphenicol, various antipyretics, and analgesics. The

degree of haemolysis depends on the type and dose of the drug involved. Patients suffering from impaired liver and kidney function are more subject to haemolysis

half-life — Often abbreviated to $\frac{T}{2}$ or $T\frac{1}{2}$. The time required for a concentration or an effect to decline by one-half. It can be measured in three ways: (*a*) by plasma half-life; (*b*) by elimination half-life, and (*c*) by biological-effect half-life.

immunity — All natural processes that prevent infection, reinfection, or superinfection, or that assist in destroying parasites or in limiting their multiplication, or that reduce the clinical effects of infection. Immunity may be natural (inherent) and independent of previous infection—for example, man is naturally immune to avian malaria, or it may be acquired, either passively or actively. In the latter case, it is the result of an established infection. See also under *semi-immune*.

incubation period — 1. The time elapsing between the initial malarial infection in man and the first clinical manifestations. When the time is extended to many times its normal length it is known as a protracted incubation period; this may happen in *Plasmodium vivax* infections occurring in the autumn in some temperate climates, so that the infected person may show no clinical signs until the following spring 2. Time needed for the completion of sporogony in the mosquito up to the infective stage (known as the extrinsic incubation period).

infection — Entrance, establishment, or maintenance in a host of a parasite, generally involving its multiplication; also the resulting condition in the host

infectious — Capable of transmitting infection; term commonly applied to the human host.

infective — Capable of transmitting infection; term commonly applied to the parasite (e.g., gametocyte, sporozoite,) or infecting agent.

international nonproprietary names (INN) — Nonproprietary names of drugs considered by the World Health Organization and included in the official list published by it.

isolate — A sample of parasites, not necessarily genetically homogeneous, collected in nature from a host and preserved in the laboratory by passaging through other hosts or by *in vitro* culture The term is now increasingly used in preference to the common but rather loose term "strain". See also *clone*, *line*, *stabilate*, and *strain*.

isomerism — Phenomenon existing in chemical compounds with identical molecular formulae but different molecular structures. Substances that are isomeric with one another are known as isomers or isomerides. Geometrical isomerism and optical isomerism depend on the spatial distribution of the four bonds of the carbon atom in the derivatives of the benzene nucleus.

latent period — Stage during which malarial infection in the vertebrate is not evidenced clinically by any symptoms of disease; occasionally used for the condition in which few or no parasites can be detected by microscopic examination. There is normally a

latent period preceding the primary attack ("incubation latency") and a period or periods of latency between the relapses following the primary attack, when the erythrocytic forms have disappeared from the blood but infection persists.

LD_{50}/ED_5 — Abbreviation for median lethal dose/median effective dose. An experimental modification of the therapeutic index on the basis of animal data obtained in laboratory studies. It refers to the relationship between the dose lethal to 50 % of animals and the dose that is effective in a desired way in 50 % of the same animals.

line — A population of parasites that have undergone a particular laboratory passage, usually following special selection, natural or experimental, for a given characteristic (e.g., drug resistance).

malaria, benign tertian — Synonym for *vivax malaria*.

malaria, cerebral — A form of pernicious malaria associated with cerebral symptoms and due to infection with *Plasmodium falciparum*.

malaria, chronic — Colloquial term for the state of ill-health associated with prolonged and repeated malaria infection. Its use is not recommended.

malaria, falciparum — Malaria infection caused by *Plasmodium falciparum*.

malaria, imported — See under *malaria case, imported*.

malaria, induced — Malaria infection properly attributable to the effect of a blood transfusion or other form of parenteral inoculation, but not to normal transmission by the mosquito in nature. Induced malaria may occur accidentally or may be produced deliberately for therapeutic or experimental purposes.

malaria, introduced — See under *malaria case, introduced*.

malaria, malariae — Malaria infection caused by *Plasmodium malariae*. The term "quartan malaria" is preferable.

malaria, ovale — Malaria infection caused by *Plasmodium ovale*.

malaria, pernicious — Malaria infection with severe symptoms, usually due to *Plasmodium falciparum*.

malaria, quartan — Colloquial name for malaria infection caused by *Plasmodium malariae*.

malaria, relapsing — See under *malaria case, relapsing*.

malaria, subtertian — Synonym of *falciparum malaria*.

malaria, tertian — Synonym of *vivax* or *ovale malaria*.

malaria, vivax — Malaria infection caused by *Plasmodium vivax*.

malaria case — In common terminology, occurrence of malaria infection in a person in whom, regardless of the presence or absence of clinical symptoms, the evidence of malaria parasites in the blood has been obtained by microscopic examination. During surveillance, every malaria case detected is classified, according to the origin of the infection.

malaria case, imported — A case in which the infection was acquired outside the area in which it is found, implying that its origin can be traced to a known malarious area

malaria case introduced — In common terminology, a case in which it can be proved that the infection is the first step (direct secondary) of local transmission subsequent to the occurrence of a proved imported case

malaria case, relapsing — In common terminology, the classification used for a case in which the renewal of symptoms or reappearance of parasites can be related to a previous infection

malarial haemoglobinuria — See under *blackwater fever*

malariotherapy — Treatment of certain diseases, notably neurosyphilis, by deliberately infecting the patient with malaria See also under *malaria induced*

mass drug administration — Distribution of a specified drug to every member of a target population within an area or locality The frequency of distribution depends on the purpose, the nature and dosage of the drug, and the local conditions

medicated salt distribution — Distribution of common salt containing an antimalarial drug in such proportion that every user will obtain with his regular food a daily amount of the drug sufficient to eliminate malaria parasites from the blood stream Formerly known as "Pinotti's method"

metabolic disposition The way, degree and speed of absorption, the biochemical change, and the elimination of a chemical compound introduced into the organism

parasitaemia Condition in which malaria parasites are present in the blood If this condition in the human subject is not accompanied by pyrexia or other symptoms of malaria except for a possible enlargement of the spleen, it is known as asymptomatic parasitaemia, and the person exhibiting the condition is known as a symptomless parasite carrier Asymptomatic parasitaemia may be primary (occurring before primary-attack symptoms) or secondary

parasite clearance time Time elapsing from the first drug administration to the first occasion on which no parasites can be demonstrated in the blood

paroxysm Cyclic manifestation of acute illness in malaria characterized by a rise in temperature with accompanying symptoms, usually caused by invasion of the blood by a brood of erythrocytic parasites

patent period Stage during which malaria infection in the vertebrate is evidenced by the presence of parasites in the blood A subpatent period is sometimes distinguished during which parasites are believed to be present in the blood in very small numbers but are not detectable by normal microscopic examination

pharmacodynamics The branch of science devoted to studies on the biological and therapeutic effects of drugs

pharmacogenetics — The study of genetically related individual and collective (viz., ethnic) variation in the absorption of, metabolism of, and response to drugs

pharmacokinetics — The branch of science devoted to the study of the absorption, distribution, metabolism and excretion of drugs

potentiation — See under *synergism*

prepatent period — Early stage of malarial infection in the vertebrate, before the invasion of erythrocytes is microscopically detectable. The prepatent period must be distinguished from the incubation period, which is related to the first clinical manifestation of the disease

prophylaxis — Any method of protection from or prevention of disease, when applied to chemotherapy it is commonly designated as "drug prophylaxis" or "chemoprophylaxis"

prophylaxis causal — Complete prevention of erythrocytic infection by the administration of drugs that destroy either the sporozoites or the tissue forms of the malaria parasite

prophylaxis clinical — Synonym of *suppressive treatment*

proprietary name — A brand name given to a drug, a drug combination, or a drug formulation by a commercial firm that sells, but does not necessarily manufacture, the product

quartan — Recurring every third day (every 72 hours). Recurrence of symptoms on two successive days, with one-day free intervals, is known as double-quartan periodicity. See also under *malaria quartan*

recrudescence — Renewed manifestation of infection (short-term relapse) believed to be due to the survival of erythrocytic forms. Not to be confused with *recurrence*

recurrence — Renewed manifestation of infection (long-term relapse) believed to be due to reinfection of erythrocytes from exoerythrocytic forms. Not to be confused with *recrudescence*

regimen — Prescribed course of drug administration for the treatment or prevention of malaria infection

relapse — Renewed manifestation (of clinical symptoms and or parasitaemia) of malarial infection separated from previous manifestations of the same infection by an interval greater than that of the normal periodicity of the paroxysms. Relapses are sometimes classified as recrudescences and recurrences, they may be either clinical or parasitic, the latter being evidenced only by the reappearance or increase in the number of parasites in the blood. The qualifications "short-term" and "long-term" may be used to designate relapses following the primary attack after intervals of less than two or more than six months, respectively. (Note. The term "relapse" should be reserved for renewed manifestations of an infection originating from exoerythrocytic stages of the parasite. The term "relapse pattern" is used to indicate a particular sequence of relapses in a given individual.)

resistance — The ability of a parasite strain to multiply or to survive in the presence of concentrations of a drug that normally destroy

parasites of the same species or prevent their multiplication Such resistance may be relative (yielding to increased doses of the drug tolerated by the host) or complete (withstanding maximum doses tolerated by the host)

schizontocide — A drug that destroys the asexual forms of malaria parasites Schizontocides are distinguished as blood schizontocides and tissue schizontocides When the term schizontocide" is used alone it usually refers to a blood schizontocide i e , one that acts on the erythrocytic asexual parasites Tissue schizontocides are drugs that destroy the exoerythrocytic stages of the parasite If they act on the primary exoerythrocytic forms they are referred to as primary tissue schizontocides (' causal prophylactic drugs) if on the latent forms as secondary tissue schizontocides a term that is rarely used and that, strictly speaking refers to drugs effecting radical treatment

screening — Evaluation of potentially useful chemical compounds for antiplasmodial activity Primary screening usually on avian or rodent malaria determines whether a compound shows any effect against malaria parasites Secondary screening indicates the qualitative and quantitative activity and preliminary toxicity of compounds that have passed through primary screening Tertiary screening usually on lower primates determines the action of the compounds prior to their use on human beings

semi-immune — An imprecise but commonly used term referring to a degree of immunity to reinfection acquired by persons exposed to malaria in areas where the disease is highly endemic, so that the signs and symptoms of disease are very mild or limited to the presence of scanty numbers of parasites in the blood Any lengthy sojourn in non malarious areas decreases the degree of this immunity and it is likely that intercurrent disease has the same effect

side chain — A chemical group attached to one of the carbon atoms in the benzene ring in contradistinction the ring comprising the six carbon atoms and the residue of hydrogen atoms is known as a benzene nucleus or simply nucleus

species — Group of organisms capable of exchanging genetic material with one another and incapable, by reason of their genetic constitution of exchanging such material with any other group of organisms The limits of species are indicated by the comparative study of morphological and other characters The present methods of study of protozoan pathogens employ in addition to conventional morphology and electron microscopy, other intrinsic characters such as molecular composition in the form of deoxyribonucleic acid (DNA) constitution or isoenzyme typing

stabilate — A sample of parasites collected and maintained in a viable state (usually by cryopreservation) with the intention of conserving all its original characteristics

strain — A population of the same stock descended from a common ancestor or derived from a single source and maintained by serial transfers in appropriate hosts or in subcultures Strains

behaving in a similar manner are homologous, those behaving dissimilarly, heterologous In the past, the term "strain" was loosely employed to denote a closely related group of individuals that perpetuate their characters in successive generations See also *isolate*

synergism — Combined action of two or more compounds resulting in a biological effect that is either a simple additive result or greater than the latter Potentiation occurs when one drug increases the action of another drug The term *potentiation* is often loosely applied to describe the phenomenon of synergism when two compounds act on different receptor sites of the pathogen Antagonism is the opposite situation, when the actions of two or more compounds result in a decreased pharmacological effect

tertian — Recurring every other day (every 48 hours) See also *malaria tertian*

therapeutic equivalence — This is demonstrated by different pharmaceutical products which, when administered in the same regimen, give results indicating essentially the same efficacy and/or toxicity

therapeutic index — Relationship between the minimum curative dose of a drug and the maximum tolerated dose expressed as a ratio This index fails to take into account the variability seen even in the most uniform human populations it may bear little relation to the therapeutic or adverse effects occurring in some subjects and in clinical practice, cannot be calculated, especially for drugs taken over a long period However the concept embodies the relation of safety of a drug to its efficacy See also under LD_{50} ED_{50}

therapeutic trial — A scientific evaluation of a drug for specific use in the treatment of a defined disease or infection

tolerance — A term which, having acquired a new and more specific meaning in immunology now refers to the failure of an individual to develop an immune response to an antigen encountered during embryonic or post-natal life In pharmacology this term is commonly employed to denote a lessened response of the subject to a previously effective dose of a given drug or to indicate the ability to sustain the prolonged administration of a drug without undue harm

treatment anti-relapse — Treatment aimed at the prevention of relapses particularly long term relapses Synonymous with *radical treatment* resulting in radical cure

treatment presumptive — Administration of an antimalarial drug or drugs, usually in a single dose in suspected malaria cases before the results of blood examinations are available Its principal objectives are the relief of clinical symptoms and prevention of transmission

treatment radical — Treatment aimed at achieving radical cure This implies the use of drugs that act on the latent tissue stages (exoerythrocytic stages) of species of plasmodia that possess them See also under *treatment anti-relapse*

treatment suppressive — Treatment aimed at preventing or eliminating clinical symptoms and/or parasitaemia by the early destruction of erythrocytic parasites It does not necessarily prevent or eliminate the infection, and overt malaria may develop after drug withdrawal

treatment schedule Scheme of administration of a drug Also drug *regimen*

INTERNATIONAL NONPROPRIETARY NAMES AND SOME OTHER NONPROPRIETARY NAMES, PROPRIETARY NAMES, AND CODE NUMBERS FOR ANTIMALARIAL DRUGS

A. By chemical group

International nonproprietary names are distinguished by an asterisk and proprietary names (errors and omissions excepted) by an initial capital letter

quinine

Quinimax
Quinoforme (formiate)

3394 R P
SN 359
WR 2976

*mepacrine** (dihydrochloride)

Acrichin
Acrihina
Acriquine
Anofelin
Arichin
Atabrin
Atatrin
Atebrin
Chemiochin
Chinacrin
Crinodora
Erion
Haffkinine
Hepacrine
Italchina
Malaricida
Metaquine
Methoquine
Metocrin
Metoquina
Palacrin
Palusan
Pentilen
quinacrine
Tenicridine

3391 R P
dihydrochloride
SN 390
WR 1543

*mepacrine** methanesulfonate

Atebrine musonate
Quinacrine soluble
Quinocrine soluble

4-Aminoquinolines

*chloroquine** (diphosphate)

Aralen
Arechin
Avloclor
Bemaphate
Chinamine
Chlorochin
Chlorochina
Delagil
Malarex
Noroquine
Paraquine
Resochin
Resoquina
Resorchin
Sanoquin
Tanakan

*chloroquine** (sulfate)

Nivaquine
Nivaquine B

3377 R P sulfate

Feroquine	Tresochin
Gontochin	Trochin
Imagon	
Iroquine	3377 R. P., diphosphate
Klorokin	SN 7618
Luprochin	Win 214
Malaquine	WR 1544

*amodiaquine** (dihydrochloride)	*amodiaquine** (base)
Cam-aqi	amodiachin
Camoquin	Basoquin
Camoquinal	
Flavoquine	CAM-1201
Fluroquine	CAM-AQI
Miaquine	4281 R P.
	SN 10751
	WR 2977

*amopyroquine** (dihydrochloride)	*amopyroquine** (base)
Propoquin	amopyrochin
	CI-356
	PAM-780
	WR 4835

*cycloquine** (base)

Ciklochin
Halochin

8-Aminoquinolines

*primaquine** (diphosphate)

Neo-Plasmochin	4516 R. P., diphosphate
Neo-Quipenyl	SN 13272
	WR 2975

*quinocide** (dihydrochloride)

chinocid	CN 1115
	Win 10448

*pamaquine** (base)

Aminoquin	Plasmochin
Beprochin	Plasmoquine
Gamefar	Praequine
Gametocide	Proechin
Leprochin	Quipenyl
pamachin	

Other 8-aminoquinolines (modified pamaquine)

Antimalarine	Fourneau 710
Certuna	Plasmocide
Cilional	Rhodoquine

Dihydrofolate Reductase Inhibitors

*proguanil** (hydrochloride)

Balusil	Lepadina	Chloriguane
Biguanide	Paludrine	M 4888
Biguanil	Palusil	3359 R P . hydrochloride
bigumal	Plasin	SN 12837
Chlorguanide	Proguanide	WR 3091
Diguanyl	Tiriam	
Drinupal		
Guanatol		

*proguanil** (lactate)

*chlorproguanil** (hydrochloride)

Lapudrine	M 5913

*cycloguanil embonate**

Camolar	CI-501
cycloguanil pamoate	CN-14329-23A

*pyrimethamine** (base)

Chloridin	BW 50-63
Darapram	D R 16056
Daraprim	NSC 3061
Erbaprelina	4753 R P
Malocide	WR 2978
Tindurin	

*trimethoprim**

Syraprim	BW 56-72
	Ro 5-6846
	20932 R P
	WR 5949

Sulfones

*dapsone**

Avlosulfone	Diphone	PAM-1111
Croysulfone	Disulone	1358 R
Damitone	Eporal	2466 R P
Daphone	Novophone	WR 0448
DDS	Sulfadione	
Diaphenason	Udolac	
Diaphenylsulfone		
Diatox		

*acedapsone**

Camılan
Hansolar
Rodılone
Sulfadıamıne

CI-556
DADDS
1555 F
PAM-1165
SN 759

Sulfonamides

*sulfadiazine**

Adıazıne
Codıazıne
Cremodıazıne
Debenal
Dıazıne
Dıazyl
Eskadıazıne
Eustral
Keladıazıne

Pırımal
Pyrımal
Ster azine
Sulfazine

2616 R P
SN 112
WR 7557

*sulfadımethoxine**

Levisul
Madrıbon
Madrıquid
sulfadımethoxy-
pyrımıdıne

10659 R P

*sulfamethoxypyridazine**

Davosın
Deposulfal
Depovermıl
Kynex
Lederkyn
Mıdıcel
Mıdıkel

Myerul
Spofadıazıne
Sulfadurazın
Sulfalex
Sultırene
Unosulf

CL 13494
7522 R P

*sulfadoxine**

Fanasıl
Fanasulf
Fanzıl
sulformethoxıne
sulforthodımethoxıne
sulforthomıdıne

Ro 4-4393
13114 R P

*sulfalene**

Kelfizına
Kelfizıne
sulfamethapyrazıne
sulfamethoxypyrazıne
sulfametopyrazıne

11070 R P
WR 4629

Tetracycline and Derivatives

*tetracycline** (and its salts)

Achromycin	Polycycline	5598 R P
Agromicina	Purocyclina	WR 6527
Ambramicina	Sanclomycin	
Cyclomycin	Tetrabon	
Hostacyclin	Tetracyn	
Omegamycin	Tetradecin	
Panmycin		

*doxycycline** (and its salts)

Bassado	Doxytrex
Biociclina	Novelciclina
Cirenyl	Parvidoxil
Dosil	Rodomicina
Doxacin	Sincromycin
Doxilina	Vibracina
Doxipan	Vibramycin

*minocycline** (and its salts)

Minocin	WR 87781
Minocyn	
Vectrin	

Lincomycin and Derivatives

*lincomycin**

Lincocin

*clindamycin** (and its salts)

Cleocin	U 21251
	U 28508

*streptolarvcin**

Dalacin

B By alphabetical order of proprietary names, code numbers, and nonproprietary names other than international nonproprietary names

Proprietary names (errors and omissions excepted) are distinguished by an initial capital letter

Achromycin	(tetracycline)
Acrichin	(mepacrine dihydrochloride)
Acrihina	(mepacrine dihydrochloride)
Acriquine	(mepacrine dihydrochloride)
Adiazine	(sulfadiazine)
Agromicina	(tetracycline)
Ambramicina	(tetracycline)
Aminoquin	(pamaquine base)
amodiachin	(amodiaquine base)
amopyrochin	(amopyroquine base)
Anofelin	(mepacrine dihydrochloride)

Antimalarine	(pamaquine analogue)
Aralen	(chloroquine diphosphate)
Arechin	(chloroquine diphosphate)
Arichin	(mepacrine dihydrochloride)
Atabrin	(mepacrine dihydrochloride)
Atatrin	(mepacrine dihydrochloride)
Atebrin	(mepacrine dihydrochloride)
Atebrine musonate	(mepacrine methanesulfonate)
Avloclor	(chloroquine diphosphate)
Avlosulfone	(dapsone)
Balusil	(proguanil hydrochloride)
Basoquin	(amodiaquine base)
Bassado	(doxycycline)
Bemaphate	(chloroquine diphosphate)
Beprochin	(pamaquine base)
Biguanide	(proguanil hydrochloride)
Biguanil	(proguanil hydrochloride)
bigumal	(proguanil hydrochloride)
Biociclina	(doxycycline)
Cam aqi	(amodiaquine dihydrochloride)
Camilan	(acedapsone)
Camolar	(cycloguanil embonate)
Camoprim	(amodiaquine base + primaquine base)
Camoquin	(amodiaquine dihydrochloride)
Camoquinal	(amodiaquine dihydrochloride
Certuna	(pamaquine analogue)
Chemiochin	(mepacrine dihydrochloride)
Chinacrin	(mepacrine dihydrochloride)
Chinamine	(chloroquine diphosphate)
chinocid	(quinocide dihydrochloride)
chlorguanide	(proguanil hydrochloride)
Chlorguanide	(proguanil hydrochloride)
Chloridin	(pyrimethamine base)
Chloriguane	(proguanil lactate)
Chlorochin	(chloroquine diphosphate)
Chlorochina	(chloroquine diphosphate)
Ciklochin	(cycloquine base)
Cilional	(pamaquine analogue)
Cirenyl	(doxycycline)
Cleocin	(clindamycin)
Codiazine	(sulfadiazine)
Cremodiazine	(sulfadiazine)
Crinodora	(mepacrine dihydrochloride)
Croysulfone	(dapsone)
cycloguanil pamoate	(cycloguanil embonate)
Cyclomycin	(tetracycline)
cycloquine	(7-chloro-4-[3,5 bis(diethylaminomethyl)-4-hydroxyanilino] quinoline)
Dalacin	(streptovarycin)
Damitone	(dapsone)
Daphone	(dapsone)
Daraclor	(pyrimethamine + chloroquine sulfate)

Darapram	(pyrimethamine base)
Daraprim	(pyrimethamine base)
Davosin	(sulfamethoxypyridazine)
Debenal	(sulfadiazine)
Delagil	(chloroquine diphosphate)
Deposulfal	(sulfamethoxypyridazine)
Depovernil	(sulfamethoxypyridazine)
Diaphenason	(dapsone)
Diaphenylsulfone	(dapsone)
Diatox	(dapsone)
Diazine	(sulfadiazine)
Diazyl	(sulfadiazine)
Diguanyl	(proguanil hydrochloride)
Diphone	(dapsone)
Disulone	(dapsone)
Dosil	(doxycycline)
Doxacin	(doxycycline)
Doxilina	(doxycycline)
Doxipan	(doxycycline)
Doxytrex	(doxycycline)
Drinupal	(proguanil hydrochloride)
Eporal	(dapsone)
Erbaprelina	(pyrimethamine base)
Erion	(mepacrine dihydrochloride)
Eskadiazine	(sulfadiazine)
Eustral	(sulfadiazine)
Falcidar	(pyrimethamine + sulfadoxine)
Fanasil	(sulfadoxine)
Fanasulf	(sulfadoxine)
Fansidar	(pyrimethamine + sulfadoxine)
Fanzil	(sulfadoxine)
Feroquine	(chloroquine diphosphate)
Flavoquine	(amodiaquine dihydrochloride)
Fluroquine	(amodiaquine dihydrochloride)
Fourneau 710	(pamaquine analogue)
Gamefar	(pamaquine base)
Gametocide	(pamaquine base)
Gontochin	(chloroquine diphosphate)
Guanatol	(proguanil hydrochloride)
Haffkinine	(mepacrine dihydrochloride)
Halochin	(cycloquine base)
Hansolar	(acedapsone)
Hepacrine	(mepacrine dihydrochloride)
Hostacyclin	(tetracycline)
Imagon	(chloroquine diphosphate)
Iroquine	(chloroquine diphosphate)
Italchina	(mepacrine dihydrochloride)
Keladiazine	(sulfadiazine)
Kelfizina	(sulfalene)
Kelfizine	(sulfalene)
Klorokin	(chloroquine diphosphate)
Kynex	(sulfamethoxypyridazine)

Lapaquin	(chloroquine + chlorproguanil)
Lapudrine	(chlorproguanil hydrochloride)
Lederkyn	(sulfamethoxypyridazine)
Lepadina	(proguanil hydrochloride)
Leprochin	(pamaquine base)
Levisul	(sulfadimethoxine)
Lincocin	(lincomycin)
Luprochin	(chloroquine diphosphate)
Madribon	(sulfadimethoxine)
Madriquid	(sulfadimethoxine)
Malaquine	(chloroquine diphosphate)
Malarex	(chloroquine diphosphate)
Malaricida	(mepacrine dihydrochloride)
Malocide	(pyrimethamine base)
Maloprim	(pyrimethamine + dapsone)
Metakelfin	(pyrimethamine + sulfalene)
Metaquine	(mepacrine dihydrochloride)
Methoquine	(mepacrine dihydrochloride)
Metocrin	(mepacrine dihydrochloride)
Metoquina	(mepacrine dihydrochloride)
Miaquine	(amodiaquine dihydrochloride)
Midicel	(sulfamethoxypyridazine)
Midikel	(sulfamethoxypyridazine)
Minocin	(minocycline)
Minocyn	(minocycline)
Myasul	(sulfamethoxypyridazine)
Neo Plasmochin	(primaquine diphosphate)
Neo Quipenyl	(primaquine diphosphate)
Nivaquine	(chloroquine sulfate)
Nivaquine B	(chloroquine sulfate)
Noroquine	(chloroquine diphosphate)
Novelciclina	(doxycycline)
Novophone	(dapsone)
Omegamycin	(tetracycline)
Palacrin	(mepacrine dihydrochloride)
Paludrine	(proguanil hydrochloride)
Palusan	(mepacrine dihydrochloride)
Palusil	(proguanil hydrochloride)
pamachin	(pamaquine base)
Panmycin	(tetracycline)
Paraquine	(chloroquine diphosphate)
Parvidoxil	(doxycycline)
Pentilen	(mepacrine dihydrochloride)
Pirimal	(sulfadiazine)
Plasin	(proguanil hydrochloride)
Plasmochin	(pamaquine base)
Plasmocide	(pamaquine analogue)
Plasmoquine	(pamaquine base)
Polycycline	(tetracycline)
Praequine	(pamaquine base)
Premaline	(chloroquine + pamaquine analogue)
Proechin	(pamaquine base)
Proguanide	(proguanil hydrochloride)

Propoquin	(amopyroquine dihydrochloride)
Purocyclina	(tetracycline)
Pyrimal	(sulfadiazine)
quinacrine	(mepacrine dihydrochloride)
Quinacrine soluble	(mepacrine methanesulfonate)
Quinimax	(quinine and other Cinchona alkaloids)
quinine	(6-methoxy-α-(5-vinyl-2-quinuclidinyl)-4-quinolinemethanol)
Quinocrine soluble	(mepacrine methanesulfonate
Quinoforme	(quinine formiate)
Quipenyl	(pamaquine base)
Resochin	(chloroquine diphosphate)
Resoquine	(chloroquine diphosphate)
Resorchin	(chloroquine diphosphate)
Rhodopraequine	(pamaquine + pamaquine analogue)
Rhodoquine	(pamaquine analogue)
Rodilone	(acedapsone)
Rodomicina	(doxycyline)
Sanclomycin	(tetracycline)
Sanoquin	(chloroquine diphosphate)
Sincromycin	(doxycycline)
Spofadiazine	(sulfamethoxypyridazine)
Sterazine	(sulfadiazine)
Sulfadiamine	(acedapsone)
sulfadimethoxypyrimidine	(sulfadimethoxine)
Sulfadione	(dapsone)
Sulfadurazin	(sulfamethoxypyridazine)
Sulfalex	(sulfamethoxypyridazine)
sulfamethapyrazine	(sulfalene)
sulfamethoxypyrazine	(sulfalene)
sulfametopyrazine	(sulfalene)
Sulfazine	(sulfadiazine)
sulformethoxine	(sulfadoxine)
sulforthodimethoxine	(sulfadoxine)
sulforthomidine	(sulfadoxine)
Sultirene	(sulfamethoxypyridazine)
Syraprim	(trimethoprim)
Tanakan	(chloroquine diphosphate)
Tenicridine	(mepacrine dihydrochloride)
Tetrabon	(tetracycline)
Tetracyn	(tetracycline)
Tetradecin	(tetracycline)
Tindurin	(pyrimethamine base)
Tiriam	(proguanil hydrochloride)
Tresochin	(chloroquine diphosphate)
Trochin	(chloroquine diphosphate)
Udolac	(dapsone)
Unosulf	(sulfamethoxypyridazine)
Vectrin	(minocycline)
Vibracina	(doxycycline)

Vibramycin	(doxycycline)
BW 50-63	(pyrimethamine base)
BW 56-72	(trimethoprim)
CAM-1201	(amodiaquine base)
CAM-AQI	(amodiaquine base)
CI-356	(amopyroquine dihydrochloride)
CI-501	(cycloguanil embonate)
CI-556	(acedapsone)
CL 13494	(sulfamethoxypyridazine)
CN 1115	(quinocide dihydrochloride)
CN-14329-23A	(cycloguanil embonate)
DADDS	(acedapsone)
DDS	(dapsone)
D R 16056	(pyrimethamine base)
1555 F	(acedapsone)
Fourneau 710	(pamaquine analogue)
M 4888	(proguanil hydrochloride)
M 5913	(chlorproguanil hydrochloride)
NSC 3061	(pyrimethamine base)
PAM-780	(amopyroquine dihydrochloride)
PAM-1111	(dapsone)
PAM 1165	(acedapsone)
1358 R	(dapsone)
Ro 4-4393	(sulfadoxine)
Ro 5-6846	(pyrimethamine base)
2466 R P	(dapsone)
2616 R P	(sulfadiazine)
3359 R P	(proguanil hydrochloride)
3377 R P	(chloroquine diphosphate and chloroquine sulfate)
3391 R P	(mepacrine dihydrochloride)
3394 R P	(quinine)
4281 R P	(amodiaquine base)
4516 R P	(primaquine diphosphate)
4753 R P	(pyrimethamine base)
5598 R P	(tetracycline)
7522 R P	(sulfamethoxypyridazine)
10659 R P	(sulfadimethoxine)
11070 R P	(sulfalene)
13114 R P	(sulfadoxine)
20932 R P	(trimethoprim)
SN 112	(sulfadiazine)
SN 359	(quinine)
SN 390	(mepacrine dihydrochloride)
SN 759	(acedapsone)
SN 7618	(chloroquine diphosphate)
SN 10751	(amodiaquine dihydrochloride)
SN 12837	(proguanil hydrochloride)
SN 13272	(primaquine diphosphate)
U-21251	(clindamycin)
U-28508	(clindamycin)
Win 214	(chloroquine diphosphate)
Win 10448	(quinocide dihydrochloride)
WR 0448	(dapsone)
WR 1543	(mepacrine dihydrochloride)
WR 1544	(chloroquine diphosphate)
WR 2975	(primaquine diphosphate)

WR 2976	(quinine)
WR 2977	(amodiaquine dihydrochloride)
WR 2978	(pyrimethamine base)
WR 3091	(proguanil hydrochloride)
WR 4629	(sulfalene)
WR 4835	(amopyroquine dihydrochloride)
WR 5949	(trimethoprim)
WR 6527	(tetracycline)
WR 7557	(sulfadiazine)
WR 87781	(minocycline)

ANNEX 3

USUAL FORMULATIONS OF ANTIMALARIAL DRUGS

1 *4-Aminoquinolines*	
(a) Amodiaquine	tablets containing 261 mg amodiaquine dihydrochloride dihydrate, corresponding to 200 mg amodiaquine base
(b) Chloroquine	tablets containing 250 mg chloroquine diphosphate, corresponding to 150 mg chloroquine base tablets containing 500 mg chloroquine diphosphate, corresponding to 300 mg chloroquine base tablets containing 136 mg chloroquine sulfate corresponding to 100 mg chloroquine base tablets containing 204 mg chloroquine sulfate, corresponding to 150 mg chloroquine base tablets containing 408 mg chloroquine sulfate, corresponding to 300 mg chloroquine base ampoules containing 5 ml chloroquine hydrochloride, diphosphate or sulfate solution at a base content of 40 mg chloroquine per ml
2 *8-Aminoquinolines*	
(a) Pamaquine	tablets containing 18 mg pamaquine naphthoate corresponding to 8 mg pamaquine base tablets containing 10 mg pamaquine dihydrochloride, corresponding to 8 mg pamaquine base tablets containing 9 mg pamaquine monohydrochloride, corresponding to 8 mg pamaquine base
(b) Primaquine	tablets containing 13 2 mg primaquine diphosphate, corresponding to 7 5 mg primaquine base
3 *Mepacrine*	tablets containing 100 mg mepacrine dihydrochloride dihydrate, corresponding to 78 5 mg mepacrine base
4 *Proguanil*	tablets of 25 mg proguanil monohydrochloride, corresponding to 22 mg proguanil base tablets of 100 mg proguanil monohydrochloride, corresponding to 87 mg proguanil base
5 *Pyrimethamine*	tablets of 25 mg pyrimethamine syrup containing 6 25 mg pyrimethamine/5 ml
6 *Quinine*[1]	tablets containing 300 mg quinine dihydrochloride, quinine hydrochloride, quinine bisulfate or quinine sulfate

[1] Among the proprietary formulations of quinine, Quinimax in the form of intramuscular injections has had some popularity in the past and is still used by a number of physicians Quinimax is available in ampoules of 4 ml, each of which contains 385 mg quinine-resorcin bichlorhydrate, 10 mg quinidine-resorcin bichlorhydrate, 2 7 mg cinchonin-resorcin bichlorhydrate, and 2 7 mg cinchonidin-resorcin bichlorhydrate

		– tablets containing 200 mg quinine sulfate – tablets containing 125 mg quinine sulfate – capsules containing 300 mg quinine sulfate – capsules containing 200 mg quinine sulfate – capsules containing 125 mg quinine sulfate ampoules containing 500 mg quinine dihydrochloride in 1 ml bidistilled water ampoules containing 600 mg quinine dihydrochloride in 2 ml bidistilled water – ampoules containing 1000 mg quinine dihydrochloride in 2 ml bidistilled water
7	*Sulfadoxine*	– tablets containing 500 mg sulfadoxine ampoules containing 1 g sulfadoxine in 4 ml injectable solution
8	*Sulfadoxine/pyrimethamine*	tablets containing 500 mg sulfadoxine and 25 mg pyrimethamine ampoules containing 500 mg sulfadoxine and 25 mg pyrimethamine in 2.5 ml injectable solution
9	*Sulfalene/pyrimethamine*	tablets containing 500 mg sulfalene and 25 mg pyrimethamine
10	*Dapsone/pyrimethamine*	tablets containing 100 mg dapsone and 12.5 mg pyrimethamine
11	*Tetracycline*	capsules containing 200 or 250 mg tetracycline hydrochloride
	Doxycycline	capsules containing 100 mg
	Minocycline	tablets containing 108 mg minocycline hydrochloride, corresponding to 100 mg minocycline base
12	*Lincomycin*	capsules containing 500 mg of lincomycin hydrochloride
	Clindamycin	capsules containing 150 mg

ANNEX 4

TESTS FOR THE PRESENCE OF ANTIMALARIALS IN BIOLOGICAL FLUIDS[1]

CHLOROQUINE

A double extraction procedure for the estimation of chloroquine in body fluids has been worked out by Brodie et al (1947) [2]

Reagents

Standard solution of chloroquine, 100 mg per litre 161 mg of the diphosphate salt are dissolved in 1 litre of HCl (0 1 mol/l) This solution is stable when stored in a refrigerator Working standards are prepared daily by dilution with HCl (0 1 mol/l)

Sodium hydroxide, NaOH (0 1 mol/l)

Heptane a technical grade of heptane is purified by successive washings with NaOH (0 1 mol/l), HCl (0 1 mol/l), and water

Absolute ethanol

Hydrochloric acid, HCl (0 1 mol/l)

NaOH (0 5 mol/l) 1 ml should be neutralized by 5 ml of the above HCl (0 1 mol/l)

Buffer reagent, pH 9 5 5 volumes of boric acid (0 6 mol/l) in potassium chloride KCl (0 6 mol/l) are added to 3 2 volumes of NaOH (0 6 mol/l) When diluted as described in the procedure, and after the addition of the cysteine reagent, the resulting pH should be 9 4 9 6 This pH should be checked by direct measurement

Cysteine reagent (50 g/1) 1000 mg cysteine hydrochloride are dissolved in 20 ml water This solution is neutralized by the addition of 0 8 ml NaOH (10 mol/l) This reagent should be made fresh daily and neutralized just before use

---

[1] The techniques described in this Annex can be used either in the field or in a reasonably well equipped base laboratory Other tests that require specialized and highly sophisticated equipment are not described here in detail

[2] A modification of this method was published by McChesney et al (1956)

Procedure

(1) Add 1–10 ml of biological sample (containing up to 1 μg chloroquine) and an equal volume of NaOH (0.1 mol/l) to 30 ml heptane in a 60-ml glass-stoppered bottle.

(2) Shake for 30 minutes. Allow the phases to separate, centrifuging if necessary.

(3) Add 8 drops of ethanol and mix with the heptane phase so as not to disturb the aqueous phase.

(4) Transfer the heptane to a 125-ml glass-stoppered bottle, add about twice the volume of NaOH (0.1 mol/l), and shake for 5 minutes.

(5) After settling, add 8 drops of ethanol to the heptane and mix as before. Remove the aqueous phase by aspiration.

(6) Repeat the washing with NaOH (0.1 mol/l), add 5 drops of ethanol, and transfer 20 ml of the heptane to a 60-ml glass-stoppered bottle containing 6 ml HCl (0.1 mol/l).

(7) Shake for 3 minutes and then centrifuge for 2 minutes.

(8) Transfer 5 ml of the acid phase to a fluorometer tube containing 1.0 ml NaOH (0.5 mol/l) and 1.5 ml buffer reagent. Add 0.5 ml cysteine reagent and mix.

(9) Run a reagent blank through the same procedure. with water substituted for plasma or urine.

Prepare standards by adding known amounts of the drug in 5 ml HCl (0.1 mol/l) to 1 ml NaOH (0.5 mol/l) and 1.5 ml buffer in fluorometer tubes. Add 0.5 ml cysteine reagent to each tube. Use dummy consisting of acid, alkali, buffer and cysteine reagent for the blank setting of the fluorometer. After 30 minutes irradiate all the tubes with ultraviolet light, as described below.

The intensity of fluorescence of the irradiated samples is determined in the Coleman photofluorometer, using Coleman B_1S and Coleman PC_1 filters. The sensitivity of the instrument is set by quinine, since the irradiated standards may slowly lose their fluorescence on frequent exposure to the ultraviolet light of the fluorometer.

Irradiation of the 4-aminoquinolines is effected in a simply constructed irradiator, using an H–4 mercury arc lamp as the light source. The samples are placed in a circular rack surrounding the lamp so that they are equidistant from the lamp, and are irradiated for 3 hours. A fan is used for keeping the temperature of the solutions below 35 °C during irradiation.

Simple field tests for the qualitative determination of chloroquine in urine have been described.

A. Wilson & Edeson Test (1954)

Reagents

Mercuric chloride ($HgCl_2$)	6.75 g
Potassium iodide (KI)	25.0 g
Distilled water	500 ml

Procedure

(1) Dissolve the $HgCl_2$ in 375 ml and the KI in 100 ml of distilled water. Pour the first solution into the second, shaking the recipient, and make up to 500 ml. This solution is known as Mayer-Tanret reagent.

(2) Add a few drops of this reagent to 5 ml cold urine in a test-tube (standing the specimen in a refrigerator for 30 minutes before testing makes the test more sensitive).

(3) The appearance of a white turbidity that clears on heating and reappears on cooling is indicative of the presence of chloroquine in the specimen. If the turbidity increases on heating albumin may be present.

(4) If the presence of albumin is suspected, boil and filter the urine, then cool in a refrigerator before adding the Mayer-Tanret reagent.

This test becomes positive within 12 hours after administering a single dose of 600 mg chloroquine base by mouth and remains positive in most subjects for 5-6 days. It indicates a urine content of 0.4-1.0 mg/100 ml or above. Other basic drugs that may give a false positive reaction in this test include quinine, primaquine, codeine, ephedrine and pethidine.

B. Lelijveld & Kortmann Test (1970)

This test is based on an unpublished procedure designed by W. A. Dill & A. J. Glazko for the determination of amodiaquine, for which it may also be employed.

Reagents

Eosin powder	50 mg
Reagent grade chloroform	100 ml
Hydrochloric acid, HCl (1 mol/l)	

Procedure

(1) Add the 50 mg eosin to the 100 ml chloroform and 1 ml HCl (1 mol/l) in a glass-stoppered separating funnel.

(2) Shake gently for a few minutes until the chloroform becomes light yellow in colour.

(3) Separate the chloroform layer and store in a dry brown glass-stoppered bottle.

(4) Add 10 drops of the chloroform solution to 2 ml urine in a test-tube and mix vigorously for a few moments.

(5) The presence of chloroquine in the urine is indicated by a change in the colour of the precipitated chloroform layer from light yellow to violet-red.

The test is positive after as small an adult dosage as 150 mg chloroquine base, and can be used for urines that fail to clear after prior boiling and filtration. For doses of 5 mg chloroquine base per kg of body weight, this procedure is reliable only up to 48 hours after drug administration.

C. Haskin Test

Reagents

Sodium hydroxide solution, NaOH (100 g/l)
Chloroform or ethylene dichloride, purified
Methyl orange solution (prepared by dissolving 0.1 g methyl orange, indicator grade, in 100 ml 5 % boric acid solution). Shake the mixture for a few hours or overnight and filter it. The clear filtrate is used as a reagent. If a precipitate forms it can be removed by filtration without loss of efficiency. This reagent is stable for some time.

Procedure

(1) Add 1 ml sodium hydroxide solution and 5 ml chloroform to a test-tube containing 5 ml urine.

(2) Cork the tube and shake for 1 minute. Allow the layers to separate (centrifugation may be necessary).

(3) Pipette the supernatant layer and transfer the chloroform carefully to a clean tube.

(4) Add 0.5 ml of the methyl orange solution to the chloroform, cork the tube and shake for 20 seconds. Allow the layers to separate.

Urine containing 0.2 mg chloroquine per 100 ml gives a perceptible reaction. An amount of 0.5 mg per 100 ml gives a definite yellow colour and 1.0 mg per 100 ml gives an intense yellow colour. This test becomes positive within 4–5 hours after the administration of a single dose of 300 mg chloroquine base; it remains positive for 4–5 days and slowly declines in intensity until it becomes indistinguishable from the blank on the tenth day.

AMODIAQUINE

Amodiaquine in body fluids may be measured by the fluorometric method of Trenholme et al. (1974).

Reagents

1,2-dichlorethane, purified for fluorometric use by successive washing with sodium hydroxide NaOH (1 mol/l), hydrochloric acid HCl (1 mol/l) and distilled water.

Borate buffer containing 6 parts of NaOH (0.6 mol/l), and 5 parts of boric acid (0.6 mol/l) in KCl (0.6 mol/l).

Dipotassium hydrogen phosphate solution (500 g/l).

HCl (0.1 mol/l).

Amodiaquine hydrochloride dihydrate, pure, for preparation of standard solution.

Procedure

(1) For serum analysis collect blood without anticoagulant; for the determination of red cell levels use tubes containing sodium oxalate.

(2) Centrifuge whole blood, remove the plasma and buffy coat, and dilute the packed red cells 1:1 with distilled water for ease of handling.

(3) Place 2 ml of serum (plain or oxalated) or of the diluted red cells in a 15-ml glass-stoppered conical tube.

(4) Add 0.2 ml of dipotassium hydrogen phosphate (K_2HPO_3) solution.

(5) Add 10 ml 1,2-dichlorethane and agitate on a mechanical shaker for 30 minutes at room temperature.

(6) Centrifuge at 1500 *g* for 10 minutes at room temperature.

(7) Discard the upper (plasma) layer and any semi-solid material that may form above the lower dichlorethane layer.

(8) Transfer 8 ml of the 1,2-dichlorethane layer to a clean 15-ml conical tube containing 3 ml HCl (0.1 mol/l) Shake at room temperature for 15 minutes.

(9) Allow the 2 liquid phases to separate, then transfer 2 ml of the upper acid phase to a test-tube containing 1 ml borate buffer. (The pH of the resultant mixture should be 9.5.)

(10) Cap with a loose glass or marble stopper, then stand in a boiling water bath for 30 minutes.

(11) Remove from the water bath and stand at room temperature for 20 minutes.

(12) Transfer 2 ml to a quartz cuvette and measure the emission fluorescence in a suitable spectrofluorometer at 390 nm, using an excitation wavelength of 250 nm.

(13) Prepare standard curves after adding known amounts of amodiaquine to samples of serum or packed red cells The lower limit of linearity for these curves is in the region of 50 μg/litre (The nature of the fluorescent derivatives of amodiaquine produced by heating in alkaline solution in the course of this procedure is unknown)

QUININE

Quinine may be detected in the urine using the Mayer-Tanret reagent described on page 195 for chloroquine

Mayer-Tanret reagent

Mercuric chloride, $HgCl_2$	6 75 g
Potassium iodide, KI	25 0 g
Distilled water	500 ml

Procedure

(1) Add clear urine to each of 2 test-tubes (cloudy urine should be shaken with kieselguhr and filtered)

(2) Add a few drops of acetic acid to one tube

(3) Add a few drops of the reagent to both tubes

Interpretation

(1) If both tubes remain clear the test is negative

(2) If both tubes show turbidity the presence of quinine is probable Confirm by demonstrating the disappearance of turbidity on boiling the acidified urine in tube 2 Should the urine remain turbid the presence of albumin is probable Filter hot to remove the albumin, quinine precipitates as the filtrate cools

(3) If the acid tube alone shows turbidity, the presence of quinine or albumin or both may be inferred To identify quinine, boil and filter hot, as in (2) Precipitates attributable to quinine and albumin appear almost immediately Sometimes turbidity develops slowly in the acid tube, when the presence both of quinine and of albumin can be excluded Precipitates that appear late may be ignored for the purpose of the test

The following procedure for the estimation of quinine in plasma or urine is that described by Hall et al (1973), which is based on the benzene extraction technique of Brodie et al (1947) It measures quinine itself, but not its metabolites

Reagents

Sodium hydroxide, NaOH (0 1 mol l)
Analytical reagent quality benzene
Sulfuric acid, H_2SO_4 (0 05 mol l)
Quinine dihydrochloride dissolves readily in cold water forming a stable solution, 125 mg salt being equivalent to 100 mg base A stock solution containing 125 mg in 100 ml water can be diluted with fresh plasma or urine to provide the standards referred to in (7) and (8) below

Procedure

(1) Add 0 5 ml plasma to 1 0 ml sodium hydroxide (0 1 mol/l) drop by drop with shaking

(2) Then add 7 5 ml benzene with further shaking for 5 minutes

(3) Centrifuge the mixture at 3000 rev min for 10 minutes

(4) Transfer 5 ml of the top (benzene) layer to another tube

(5) Add 5 ml sulfuric acid (0 05 mol l) and shake the mixture vigorously for 5 minutes

(6) Discard the benzene layer and use the remaining layer for quinine measurements

(7) Make duplicate readings on a spectrofluorometer at 350 nm activation and 450 nm fluorescence Use 3 fresh plasma standards containing 2 5, 5 0 and 7 5 mg/l quinine base and a fresh plasma blank with each set of determinations

(8) When determining urine quinine levels follow the same procedure but use standards containing 2 5 10 and 20 mg/l quinine base and a urine blank

MEPACRINE

The single extraction procedure of Brodie et al (1947) is recommended for the estimation of mepacrine in body fluids A modification of the test has been found useful for the detection of mepacrine in urine

(1) To 10 ml urine in a test-tube add 1 ml saturated solution of potassium bicarbonate

(2) To the alkalized urine add 0 25 ml amyl alcohol

(3) Thoroughly shake and put aside for a few minutes to allow the alcohol to separate

(4) Examine by ultraviolet light for yellow fluorescence in the alcohol layer

PROGUANIL

A simple test for proguanil in the urine has been devised by Gage & Rose (1946)

Reagents

Copper sulfate analytical reagent (AR), 500 mg, and ammonium chloride AR, 1.330 mg, in 100 ml distilled water
Sodium diethyldithiocarbamate: 0.1 % aqueous solution (the solution does not keep for more than a fortnight)
Sodium hydroxide (1.0 mol/l)
Benzene: the commercial grade, redistilled, is satisfactory.

Procedure

(1) Mix 2 ml urine, 1 ml copper reagent, and 1 ml sodium hydroxide solution in a stoppered tube and allow to stand for a few minutes.

(2) Add 5 ml benzene and shake for 2 minutes. Decant or pipette off the benzene into a second tube, wash with 1 ml water, and transfer to a third tube.

(3) Shake with 1 ml sodium diethyldithiocarbamate solution for 1 minute.

The intensity of the golden-yellow colour is a measure of the amount of proguanil for comparison with a prepared set of standards

PYRIMETHAMINE

At recommended dosages the plasma and urine levels are very low, necessitating the use of specialized analytical facilities for their chemical determination. Jones & King (1968) have indicated a relatively simple qualitative procedure for urine by thin-layer chromatography, but gas or high pressure liquid chromatography is required for quantitative analysis (Jones & Ovenell, 1979). The bioassay procedure described by Richards & Maples (1979) is particularly useful as it gives a direct measure of the level of activity of pyrimethamine in plasma against asexual stages of *P. falciparum* (see Fig. 20, page 78).

PRIMAQUINE

Because of the rapidity of elimination and the low dosage administered, sophisticated laboratory procedures such as gas chromatography and mass spectrometry are required to determine plasma concentrations. As these are available only in specialized research laboratories technical details are not presented here.

SULFONAMIDES AND SULFONES

A. Sulfonamides

The concentration of free and acetylated sulfonamides in urine or plasma can be determined by the Bratton–Marshall technique (1939).

Reagents

A solution of 100 mg *N*-(1-naphthyl) ethylenediamine dihydrochloride pure substance in 100 ml water (this reagent to be kept in a dark bottle).
Aqueous sodium nitrite solution (1 g/l)
A solution of 15 g trichloracetic acid in 100 ml water
Saponin 0.5 g/l water
Hydrochloric acid, HCl (4 mol/l)
Ammonium sulfamate, 0.5 g/100 ml water
Stock solution of sulfanilamide (or other reference sulfonamide), 200 mg/l

Procedure for blood

(1) Make reference solutions from the stock sulfonamide solution by adding 5, 2.5 and 1 ml to 18 ml of the 15 % trichloracetic acid solution and diluting to 100 ml. This yields concentrations of 1 0.5 and 0.2 mg sulfonamide per 100 ml.

(2) Dilute 2 ml oxalated blood with 30 ml saponin solution.

(3) After leaving to stand for 2–3 minutes, precipitate with 8 ml of the trichloracetic acid solution. Filter.

(4) To 10 ml filtrate add 1 ml sodium nitrite solution. Leave to stand for 3 minutes.

(5) Add 1 ml sulfamate solution. Leave to stand for 2 minutes.

(6) Add 1 ml *N*-(1-naphthyl) ethylenediamine hydrochloride solution.

(7) Compare the purple-red colour reaction in a suitable colorimeter with the standard solution. This gives a value for the *free sulfonamide* in the specimen. (The colour remains stable up to 1 hour.)

(8) Add 0.5 ml HCl (4 mol/l) to 10 ml filtrate, heat in a boiling water bath for 1 hour, cool, adjust the volume to 10 ml.

(9) Repeat steps (4) to (7) above to obtain a value for the *total sulfonamide*.

If a photoelectric colorimeter is used the blood can be diluted 1:50 or 1:100 with distilled water, no saponin being required. Trichloracetic acid is added to a volume which is 1/5 that of the final mixture. This allows determinations to be made on as little as 0.1- or 0.2-ml samples of blood. A suitable filter must be used in the colorimeter as the peak of absorption of the azo dye is at 545 nm.

2. *Procedure for urine* (Bratton–Marshall technique)

(1) Add to 50 ml urine 5 ml HCl (4 mol/l) and dilute to 100 ml.

(2) Using 10-ml aliquots, follow steps (4) to (7) in the procedure for blood to determine the quantity of *free sulfonamide*.

(3) Boil 10 ml diluted acidified urine (step (1) above), then proceed as in steps (8) and (9) of the procedure for blood to determine the levels of *total sulfonamide* (i.e., including acetylated compound).

If the urine contains protein, proceed with step (1) above, then precipitate protein with trichloracetic acid as in step (3) of the procedure for blood before continuing to (4), etc. of that procedure. Minor modifications in this procedure relating to specific sulfonamides have been published by various authors, but the basic Bratton–Marshall procedure appears to be applicable to all those in general use. (Urine that proves to contain more than 10–20 mg sulfonamide per litre should be first diluted to bring it down to this level, and the procedure in this section carried out on this diluted material.)

3. Procedure for urine (Lignin test)

This is a simple qualitative field test for the detection of sulfonamides in urine.

Reagents

Paper towel or blank newspaper strips
Hydrochloric acid, HCl (3 mol l)

Procedure

(1) Place one or two drops of urine on a blank strip of newspaper or paper towel.

(2) Add a small drop of HCl (3 mol/l) to the centre of the moistened area. The immediate appearance of a yellow to orange colour indicates the presence of a sulfonamide compound The test usually becomes positive 1 hour after the ingestion of sulfonamides and stays positive for 3 days. Note: paper of bond quality or filter paper cannot be used.

B. Sulfones

The procedure that follows, which was described by Glazko et al. (1968), measures microgram quantities of dapsone in plasma or urine.

Reagents

Sodium hydroxide, NaOH (1 mol/l)
1,2-dichlorethane
Hydrochloric acid, HCl (1 mol/l)
Trisodium citrate (1 mol/l)
Ethyl acetate
Anhydrous sodium sulfate
Pure dapsone for preparation of aqueous standards.

1. Procedure for blood plasma

(1) Transfer 1–3 ml plasma into 12-ml glass-stoppered conical centrifuge tubes.

(2) Add 0.1 ml of NaOH (1 mol/l) for each 1 ml plasma, and 7 ml 1,2-dichlorethane.

(3) Stopper, agitate mechanically for 15 minutes, then centrifuge briefly to separate the solvent phases

(4) Transfer 6 ml of the 1,2-dichlorethane layer to a clean centrifuge tube containing 2 5 ml HCl (1 mol l)

(5) Stopper, agitate for 5 minutes and centrifuge

(6) Transfer 2 ml of the acid layer to 1 ml trisodium citrate (1 mol/l) in a 12 × 100-mm glass-stoppered test-tube

(7) Add 2 ml ethyl acetate, agitate for 5 minutes, then centrifuge to separate the layers

(8) Transfer as much of the organic solvent layer as possible to a clean test-tube and add 1 g anhydrous sodium sulfate *slowly* with rapid agitation to prevent the salt from clumping

(9) Stopper and agitate mechanically for a few minutes to ensure that all the water is removed from the ethyl acetate

(10) Decant the solution into quartz cuvettes and compare the fluorescence at 340 nm with standard aqueous solution at 297 nm activation Levels of dapsone down to about 3 mg/l can be determined

2 Procedure for urine

The following additional *reagents* are needed.
Sodium thiosulfate ($Na_2S_2O_3$ $5H_2O$)
Hydrochloric acid, HCl (6 mol/l)
Sodium hydroxide, NaOH (500 g l)

Procedure

(1) To 1 ml urine in a 12-ml glass-stoppered centrifuge tube add 100 mg sodium thiosulfate and 0 5 ml HCl (6 mol l)

(2) Heat in a boiling water bath for 1 hour

(3) After cooling to room temperature, add 0 2 ml NaOH (500 g/l)

(4) Extract the dapsone by shaking with 6 ml 1,2-dichlorethane.

(5) Separate the layers by centrifuging, then transfer 5 ml of the 1,2-dichlorethane solution to a clean conical tube.

(6) Add 3 ml HCl (1 mol/l) and agitate for 5 minutes

(7) Transfer 2 ml of the acid layer to a 12 × 100-mm test-tube, then add 1 ml trisodium citrate (1 mol/l)

(8) Add 2 ml ethyl acetate and agitate. Continue as in steps (8) to (9) of Procedure 1, using dapsone solutions in urine as standards instead of aqueous solutions.

POTENTIATING DRUG COMBINATIONS

See individual components. High-pressure liquid chromatography or other sophisticated chemical techniques may be necessary to estimate the extremely low levels of some of these compounds, especially when administered as repository formulations.

ANNEX 5

PROCEDURES FOR ASSESSING THE RESPONSE OF MALARIA PARASITES TO DRUGS *IN VIVO*[1]

The first step in assessing drug response is to collect baseline data on the sensitivity of *P falciparum* to chloroquine not only in localities from which reports of suspected resistance have been received but also from areas of distribution of this parasite where the drug response appears to be normal Several tests are available Selection of the appropriate one should take into account the level of immunity of the subjects to be tested, their clinical condition, and the period of time within which they can reasonably be followed up A further factor to be considered is the local epidemiological situation that will determine the likelihood of the subjects' becoming reinfected during the course of the observation period The options available are the following

(1) The WHO Standard Field Test consisting of the administration of 25 mg of chloroquine base per kilogram of body weight over 3 days, with a 7-day observation period (sometimes referred to as the "7-day test")

(2) The same test with the observation period extended to a total of 28 days, referred to as the 'extended test"

(3) The single-dose test or "alternative test", consisting of the administration of 10 mg of chloroquine base per kilogram of body weight This test is applicable

(*a*) where for any reason treatment cannot be pursued for 3 days,

(*b*) in areas of high endemicity where, owing to the elevated level of immunity in the population, a single dose of chloroquine has been accepted as the standard form of treatment or

(*c*) as a preliminary screening procedure prior to applying the standard 3-day treatment

All these procedures are designed for use under field conditions, although the extended test often presents difficulties in the field They all give an indication of the response of the local *P falciparum* strains to the dosage of chloroquine used In principle, they should exclude various causes of drug

[1] From WHO Technical Report Series No 529 1973 (*Chemotherapy of malaria and resistance to antimalarials*) pp 32 37

failure that might otherwise lead to the erroneous belief that chloroquine resistance is present in the area

Although there is some possibility of vomiting after the first dose when chloroquine is administered by mouth, oral administration is preferred to injection because of its safety, ease, and uniformity

The test must be evaluated by the examination of thick blood films

Since transmission cannot always be excluded under field conditions, recrudescences cannot always be distinguished from reinfections A determination of resistance at the RII or RIII level is therefore based on the response of asexual parasitaemia during the first week of treatment Only if new infections can be excluded will further observations over an additional 3 weeks yield more conclusive evidence as to the recrudescence of parasitaemia, thus permitting the observer to distinguish between sensitivity (S) and the RI level of resistance

Experience has confirmed that all steps of the test must be carried out, or at least supervised, by responsible and qualified technical staff

Field test for response to a standard regimen of chloroquine

This field test may determine the response of a strain of malaria parasite to a standard test dosage of chloroquine (25 mg kg over 3 days starting on day 0) The test may be performed on subjects irrespective of age, parasite count and previous suppressive therapy However it should not be carried out on a person who is seriously ill

(1) *Procedure for standard and extended field tests*

One dose of chloroquine is given on each of 3 successive days (a total of 1 5 g of base for a 60 kg adult) according to the following schedule

day 0 first dose 10 mg kg (600 mg of base for a 60 kg adult)
day 1 second dose 10 mg kg (600 mg of base for a 60 kg adult)
day 2 third dose 5 mg kg (300 mg of base for a 60 kg adult)

The chloroquine tablets must not be coated and must comply with the standards laid down by the International Pharmacopoeia or the national pharmacopoeia of the country At the time of each drug administration precautions must be taken to ensure that the drug is swallowed and retained To avoid nausea or vomiting the drug should not be taken on an empty stomach Subjects who vomit should not be used for the test

For obvious reasons, severely ill patients should be excluded from the test Those with mixed infections should also be excluded so as to avoid confusion over species identification It is desirable where possible to include persons with high parasite counts, in practice this will mean young children in highly endemic regions

At all times the clinical condition of the patient must take precedence over the conduct of the test If the parasite count is excessively high or the patient is ill at any time, it is advisable, in areas of suspected chloroquine resistance, to administer drugs of other types, such as quinine

The subjects of the test should be observed daily for 7 days after the first day (day 0) of drug administration Even a 7 day observation period may be impracticable under field conditions but should be insisted upon if possible The standard 7-day field test does not permit the distinction between sensitivity (S) and resistance at the RI level Extended observation for an additional 21 days will usually distinguish between sensitivity and RI resistance, this is the extended field test

The results of the test may be recorded on a form such as that shown on page 209

Duplicate thick and thin blood films should be made immediately before the first test dose and repeated daily for at least 7 days, one of each set being kept for reference Parasite counts should be made and the species of parasite identified, because *P malariae* trophozoites may persist for 7 days after the start of the test procedure A thick film is considered negative when the examination of 100 fields fails to reveal any asexual parasites Whenever possible the urinary excretion of chloroquine should be determined by a suitable method

Urine should be collected prior to drug administration on day 0 or the previous day, and at least once during days 1 3 after the beginning of treatment (preferably on day 1 or 2)

The number of persons with symptomatic or asymptomatic asexual parasitaemia subjected to the test will depend upon the circumstances The test can, of course, be used individually, but if information on the baseline sensitivity of the local parasites is being sought, proper sampling methods are required to give confidence in the interpretation of the results As a working guide, tests should be made on at least 30 persons in a given locality whenever possible If a detailed search is being made for the presence or absence of resistant strains, larger numbers should be tested It is advisable that the results of blood examinations be available within 12 hours at the latest, or sooner if patients with clinical malaria are included in the test Tests carried out on partially immune asymptomatic carriers with fewer than 1000 trophozoites per mm^3 of blood probably do not provide a sound basis for the thorough assessment of the action of the drug on nonimmune subjects

(2) *Interpretation of the WHO Standard Field Test (7-day test)*

This test is interpreted as indicated in Fig 27 (see page 105)

(*a*) If no *asexual* parasites are found by day 6 and none are present on day 7, the infection may be either sensitive (S) or resistant at the RI level

(*b*) If asexual parasites disappear for at least 2 consecutive days but return and are present on day 7, they are resistant at the RI level.

(*c*) If asexual parasitaemia does not clear but is reduced to 25 % or less of the original pre-test level during the first 48 hours of treatment, the parasites are resistant at the RII level.

(*d*) If asexual parasitaemia is reduced by less than 75 % during the first 48 hours or if it continues to rise, the parasites are resistant to the standard dose of the drug at the RIII level. *Note.* Resistance at the RIII level may exist when the count on day 2 markedly exceeds the count on day 0. In this case the test should be suspended and the patient given effective treatment if his clinical condition so demands.

(3) *Interpretation of an extended test*

This test will distinguish between sensitivity (S) and the kind of resistance that is demonstrable only by recrudescence following a normal initial response. It is interpreted as follows:

(*a*) If no *asexual* parasites are found by day 6 and parasites do not reappear by day 28, the parasites are sensitive (S).

(*b*) If asexual parasites disappear as in (*a*) but return within 28 days, reinfection having been excluded, the parasites are resistant at the RI level.

(*c*) If the asexual parasitaemia does not clear but is reduced to 25 % or less of the original pre-test level during the first 48 hours of treatment, the parasites are resistant at the RII level.

(*d*) If asexual parasitaemia is reduced by less than 75 % during the first 48 hours or if it continues to rise, the parasites are resistant at the RIII level (see cautionary note under (*d*) above)

Alternative test with single-dose treatment

An alternative test using a single dose of 10 mg of chloroquine base per kilogram of body weight can be utilized instead of the 3-day regimen. The observation period is limited to 7 days and the test is interpreted as in the WHO Standard Field Test. However, should the parasitaemia fail to respond within 7 days or recrudesce during this time, the 3-day regimen should be applied *Here, too, it should be stressed that the clinical condition of the patient must at all times take precedence over the conduct of the test*

SAMPLE FORM FOR REPORTING INDIVIDUAL RESULTS OF FIELD TESTS

RESULTS OF FIELD TEST FOR STRAIN SENSITIVITY TO A STANDARD DOSE OF CHLOROQUINE IN FALCIPARUM MALARIA*

Investigator Date
Name of patient Case No
Age Sex Weight (kg) Locality
Date of first administration of drug (Day 0)
Particulars of chloroquine tablets
Brand and origin Dose of base per tablets

Day	Parasites			Drug dose (mg base)	Urine test	Remarks***
	Species	Trophozoites				
		Count**	per mm³			
– 1						
0						
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						

Name of patient Case No

Day	Parasites			Drug dose (mg base)	Urine test	Remarks***
	Species	Trophozoites				
		Count**	per mm³			
15						
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						

* If the test can be continued for 28 days blood examinations after the first week should be carried out at least twice weekly and the findings recorded similarly

** The method of counting parasites (e g per 100 fields per 100 leukocytes or per 10 000 erythrocytes) should be stated

*** Including records of oral temperature if observed

NB A statement summarizing the epidemiological situation in the area where the test was performed should accompany this report

IN VITRO TESTS FOR SUSCEPTIBILITY OF *P. FALCIPARUM* TO CHLOROQUINE AND MEFLOQUINE

The problems inherent in conducting field tests *in vivo* have underscored the need for an *in vitro* test that can be used to study the response of *P. falciparum* to various schizontocides An *in vitro* test should minimize the variations in apparent drug response due to immunity and obviate the operational difficulties of following up test subjects Such a test has been developed in the field The test procedure is as follows (Rieckmann et al , 1968)

(1) A sample of venous blood is collected into a plain silicone-coated vacuum tube, transferred immediately to a sterile Erlenmeyer flask containing glass beads, and defibrinated by rotating the flask for 5 minutes

(2) By means of a sterile 1 ml pipette, 1-ml aliquots of blood are then placed into screw-capped, flat-bottomed glass vials that contain glucose (5 mg) and either no drug (control) or the drug in various amounts

(3) Blood in the vials is swirled gently to mix the contents well, and the vials are then placed in a water bath at 38 40 C for a period of 24 hours

(4) After incubation, the vials are shaken to resuspend the cells in plasma, and thick films are prepared and stained for 20 minutes with Giemsa stain

Based on the candle-jar system for the continuous *in vitro* culture of *P falciparum* (Trager & Jensen, 1976), Rieckmann et al (1978) described a microtechnique for the assessment of drug sensitivity of *P falciparum* that offers several distinct advantages over the macrotechnique

INSTRUCTIONS FOR USE OF THE WHO TEST KIT FOR THE ASSESSMENT OF THE RESPONSE OF *P FALCIPARUM* TO CHLOROQUINE[1] AND MEFLOQUINE (Macrotechnique)

1. Test procedure (chloroquine)

Persons who have received 4-aminoquinolines within the last 14 days, or pyrimethamine and/or sulfonamides within the last 28 days, should be excluded from the test It is also advisable to subject the pre-selected patients

[1] The instructions for use of the test kit were developed on the basis of the work of Rieckmann K H & Lopez Antuñano, F J (1971) *Bull Wld Hlth Orq* , 45, 157 167 and Valera, C V & Shute, G T (unpublished document WHO/MAL 75 852)

to a urine test for chloroquine, amodiaquine, and, if so indicated, sulfonamides, and to exclude those with a positive test.

Thick and thin blood films are taken from persons suspected of having falciparum malaria. The films are Giemsa-stained and examined for malaria parasites. Blood samples should not be collected from patients with counts of less than 400 parasites/μl or over 100 000 parasites/μl, or from patients with mixed species infections. If well-developed "fleshy" rings of *Plasmodium falciparum* are observed, blood is collected for culture.

(1) Collect a sample of at least 8 ml of venous blood into the plain silicon-coated Vacutainer tube (insert needle in Vacutainer holder, puncture vein and connect Vacutainer tube by pushing up) or into a sterile disposable syringe.

(2) Transfer the blood from the tube or syringe immediately into a sterile 25-ml Erlenmeyer flask (stoppered) containing glass beads, and defibrinate by rotating the flask for 5 minutes. The blood should, if possible, be processed immediately or maintained for no longer than 3 hours at room temperature before the start of incubation [2]

(3) Using a sterile 1-ml pipette, place 1-ml aliquots of blood into the screw-capped flat-bottomed test vials in the following sequence.

In areas with suspected resistance			*In areas with no previous indication of resistance*		
2 control vials		(white)	2 control vials		(white)
chloroquine	nmol	0 50 (green)	chloroquine	nmol	0 50 (green)
,,		1 00 (grey)	,,		1 00 (grey)
,,		1 50 (blue)	,,		0 75 (orange)
,,		2 00 (black)	,,		0 25 (yellow)
,,		3 00 (red)	,,		1 50 (blue)
,,		0 25 (yellow)	,,		2 00 (black)
,,		0 75 (orange)	,,		1 25 (brown)
,,		1 25 (brown)	,,		3 00 (red)

These sequences are chosen in order to secure useful and significant results in the event that an insufficient quantity of blood should preclude the performance of the complete series, e g in children.

Vials with 4 nmol and 5 nmol chloroquine are available for special investigations

The vials are already dosed: controls with 5 mg glucose, the chloroquine vials with chloroquine + glucose. The deposit may hardly be visible as the quantities involved are minute

If more than one series is being run, it is advisable to stick round labels on the screw caps and mark them with the patient's number.

(4) Close the vials and swirl the blood in the vials gently to mix the contents

[2] If infected blood is to be held or transported over periods of 3–48 hours, it should be kept on wet ice The vial should be wrapped in gauze to avoid direct contact between ice and glass, which is liable to provoke haemolysis of the blood sample

well Then place the vials in the rack in ascending order (white, yellow, green, orange, grey, brown, blue black red as applicable)

(5) Then place the rack with the vials in a water-bath or dry-air incubator at 38 5°C for 24 hours

(6) After incubation, shake the vials to re-suspend the cells in the plasma

(7) Prepare thick films (2 for each concentration) and stain for 20 minutes with saline Giemsa stain (3 ml Giemsa stock solution diluted with 60 ml of a 9g l NaCl solution and 37 ml phosphate-buffered water, pH 6 7)

Since fresh blood has a marked tendency to become detached from the slide, it is advisable to keep one set of slides for 2 3 days prior to staining and use these slides for the final reading

(8) Then read the results as follows

Controls			*Samples with chloroquine*	
Number of schizonts (i e parasites with more than 2 nuclei) per 300 leukocytes after incubation*			Number of schizonts per 300 leukocytes after incubation ( )*	of schizonts relative to control samples (controls − 100) /) (a)
Control 1	Control 2	Mean (m)		
x	y	$m \frac{x+y}{2}$		$a \frac{-}{m} \times 100$
e g 300	340	320	64	$\frac{64}{320} \times 100 = 20(/)$

* In cases of low parasitaemia and at the higher chloroquine concentration ranges, it may be necessary to count against 1000 leukocytes

(9) Record the results in the standard test form (Fig V p 221) in the appropriate sections for chloroquine A graph sheet can be used to facilitate analysis of the findings

(10) Provided that growth in the controls is adequate enough for conclusive readings in the whole test and that the only parasites are *Plasmodium falciparum* the following conclusions can be drawn

(*a*) Total inhibition of schizont maturation at 1 00 nmol chloroquine or less (1 00 × 10 $^{-6}$ mol/l) indicates susceptibility to standard chloroquine treatment

(*b*) Schizont maturation at 1 50 nmol chloroquine or more (1 50 × 10 $^{-6}$ mol l) indicates resistance of *P falciparum* to chloroquine,

(*c*) Schizont maturation at 1 00 nmol chloroquine but inhibition at 1 50 nmol may still be compatible with a satisfactory response to chloroquine

In such cases, the use of the 1 25 nmol concentration may provide further information in conjunction with the response of the patient to chloroquine (*in*

vivo test procedure: see WHO Technical Report Series, No. 375, 1967, p. 55–59.)

N.B.: The *in vitro* test is not meant to replace the *in vivo* assessment of the response to treatment with chloroquine Together with the latter, it may provide valuable indications of parasite sensitivity and permit its monitoring.

2. Testing for the response of *Plasmodium falciparum* to mefloquine

For the *in vitro* assessment of the susceptibility of *P falciparum* to mefloquine, a special Mefloquine Test Kit has been developed to be used in conjunction with the kit for assessing the response to chloroquine

Test procedure

The test procedure is the same as in the chloroquine tests. The sequence of dosing the vials is as follows

2 control vials		(red/white)
mefloquine	nmol 0 50	(red/green)
	1 00	(red/grey)
	0 75	(red/orange)
	0 25	(red yellow)
	1 50	(red blue)
	2 00	(red/black)

Reading and recording of the results follow the procedure outlined for the chloroquine tests, but the critical level for resistance appears to be at 2 nmol (2×10^{-6} mol/l)

INSTRUCTIONS FOR USE OF THE TEST KIT FOR THE ASSESSMENT OF THE RESPONSE OF *PLASMODIUM FALCIPARUM* TO CHLOROQUINE AND MEFLOQUINE *IN VITRO* (Microtechnique)[3]

1. Lay-out of microculture plates

1 1 Chloroquine

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	
A	0	0	0	0	0	0	0	0	0	0	0	0	pmol
B	1	1	1	1	1	1	1	1	1	1	1	1	pmol
C	2	2	2	2	2	2	2	2	2	2	2	2	pmol
D	4	4	4	4	4	4	4	4	4	4	4	4	pmol
E	57	57	57	57	57	57	57	57	57	57	57	57	pmol
F	8	8	8	8	8	8	8	8	8	8	8	8	pmol
G	16	16	16	16	16	16	16	16	16	16	16	16	pmol
H	32	32	32	32	32	32	32	32	32	32	32	32	pmol

[3] The instructions for the use of the test kit were developed on the basis of the studies described by Rieckmann et al (1978) and Wernsdorfer (1980)

Well A is the control; wells B–H represent a chloroquine concentration line based on a geometrical progression of 2^0; 2^1; 2^2; 2^3; 2^4 and 2^5 pmol, with an intermediate concentration at $2^{2.5}$ (well E).

1.2 Mefloquine

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	
A	0	0	0	0	0	0	0	0	0	0	0	0	pmol
B	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	pmol
C	1	1	1	1	1	1	1	1	1	1	1	1	pmol
D	2	2	2	2	2	2	2	2	2	2	2	2	pmol
E	4	4	4	4	4	4	4	4	4	4	4	4	pmol
F	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	5.7	pmol
G	8	8	8	8	8	8	8	8	8	8	8	8	pmol
H	16	16	16	16	16	16	16	16	16	16	16	16	pmol

Well A is the control; wells B–H represent a mefloquine concentration line based on a geometrical progression of 2^{-1}; 2^0; 2^1; 2^2; 2^3 and 2^4 pmol, with an intermediate concentration at $2^{2.5}$ (well F)

Plates dosed with quinine and with amodiaquine are also available.

As the plates are pre-sealed, it is necessary to remove the seal from the columns to be used prior to placing the blood/medium mixture into wells A–H. This can be done by cutting, with a scalpel, the plastic cover sheet to the right of the last column to be used and pulling the plastic sheet from the required columns. For example, if columns 1 + 2 are to be used, the cut should be made between columns 2 and 3 and the plastic sheet is removed from columns 1 and 2.

2. Test procedure

Persons who have received 4-aminoquinolines within the last 14 days or pyrimethamine and/or sulfonamides within the last 28 days should be excluded from the test. The pre-selected patients should be subjected to a urine test for chloroquine and amodiaquine and, if so indicated, for sulfonamides, and those with a positive test should be excluded.

Thick and thin blood films are taken from persons suspected of having malaria. The films are Giemsa or Romanovsky stained and examined for malaria parasites. The *in vitro* test should not be carried out on patients with mixed infections or with counts of less than 500 asexual forms of *P. falciparum* parasites/μl. The stage of development of the asexual forms should be counted.

When the patient has been selected, the procedure is as follows:

(*a*) Remove cap from sterile holding vial (capacity approximately 5 ml).

(*b*) Inject 0.9 ml of growth medium[4] by means of a sterile tuberculin syringe into empty, sterile holding vial.

(*c*) Draw 100 μl of blood from the finger tip or earlobe (or toe in infants) in a sterile, anticoagulant-treated capillary and eject into the vial containing the growth medium Close the vial with a sterile cap and gently agitate to suspend the blood cells. Preferably process the blood/medium mixture immediately; but if need be it could be kept for up to 3 hours before adding it to the plates. However, the mixture should be kept as near to 37 C as possible Higher temperatures and cooling are to be avoided

(*d*) Remove the seal strips on the appropriate columns of the chloroquine and mefloquine plates

(*e*) Dose all the wells of the appropriate columns of the chloroquine and mefloquine plates with 50 μl of the blood/medium mixture in a descending order (starting from well A at the top, ending at well H at the lower end of the plate), using the Eppendorf pipette with a sterile tip Whenever a new concentration series is to be started, it is necessary to change the tip in order to avoid drug contamination of the control wells While dosing with the blood/medium mixture, agitate the latter gently in order to maintain the blood cells in suspension

(*f*) Place the lid on the microplate and inscribe the patient's reference number on the lid, using a glass-writing pencil.

(*g*) After the blood/medium mixture has been added, shake the culture plate gently for a few seconds to dissolve the drug deposits in the wells

(*h*) Place the culture plate in an airtight container (preferably a desiccator) with a paraffin candle (Only pure paraffin candles should be used) After the candle is lit, replace the container lid partially, leaving only a small opening. Close the lid finally just before the flame goes out If desiccators with stopcocks are used, the cover can be firmly fitted with the stopcock in the open position. It is then closed as the flame is about to go out Place the airtight container in an incubator at 37 38 C and leave there for 24 hours if the majority of rings in the pre-incubation slide were large or medium size, or for 26 hours if the majority were small

Sealed waterbaths have also given very satisfactory results Only types with slanting covers should be used, in order to avoid water dripping on the culture plates Place the plates above water level on a rack, position the candle on another and light Put the cover in place and seal Incubate at 37–38°C for 24 hours.

(*i*) After incubation, prepare 2 thick blood films from the contents of each well, after as much as possible of the supernatant has been removed by means of an ordinary capillary tube fitted with the bulb provided in the test kit. The

[4] The growth medium consists of 10.4 g RPMI 1640 (Gibco), 5.94 g HEPES (Sigma), 2.0 g sodium bicarbonate, and 50 mg gentamycin or neomycin per litre of double-distilled water

same capillary tube may be used for making the thick films, but a fresh tube must be used for each well.

Individual steps are shown in Fig. I–IV
Fig. I—preparation of the blood/medium mixture covers procedures (*a*) – (*c*)
Fig. II—dosing of microplates covers procedures (*d*) – (*g*)
Fig. III—incubation covers procedure (*h*)
Fig. IV—preparation of blood films covers procedure (*i*).

(*j*) Stain thick films for 30 minutes with Giemsa stain (2 % solution in 7.1 pH phosphate buffer).

Since culture blood has a marked tendency to become detached from the slide, it is advisable to keep the slides for 2–3 days prior to staining and use these slides for the final reading.

If faster processing is desired, dry the slides for 2 hours in the open air or in an incubator at 37°C and stain with the modified Romanovsky stain provided with the kit according to the following procedure:

(i) Determine the required amount of staining solution by calculating 2 ml per slide.

(ii) Make up staining solution by measuring the required volume of buffered water (prepared by dissolving the contents of one buffer vial in 1 litre distilled water) and by adding 4 drops of Romanovsky A and 3 drops Romanovsky B stock per 10 ml buffered water. Then stir the staining solution to assure complete mixing.

(iii) Put the slides on the curved plate, blood samples facing downwards, and carefully pour the staining solution from the side so that the space between slides and plate is completely filled up without leaving bubbles (use only freshly prepared staining solution).

(iv) After 10 minutes remove the slides and, without washing, put on a drying rack.

(*k*) The schizonts are then counted against 200 asexual parasites and the results are calculated as follows:

Controls				*Samples with chloroquine or mefloquine*	
Number of schizonts (i.e., parasites with more than 2 nuclei per 200 parasites after incubation				Number of schizonts per 200 parasites after incubation (*z*)	% of schizonts relative to control samples (controls = 100 %) (*a*)
Control 1 from chloroquine plate		Control 2 from mefloquine plate	Mean (*m*)		
	K_1	K_2	$m = \frac{K_1 + K_2}{2}$		$a = \frac{z}{m} \times 100$
e.g.	96	100	98	49	$\frac{49}{98} \times 100 = 50.0$ (%)

If there is low parasitaemia, it is permissible to count against 100 parasites only.

(*l*) The results for chloroquine are then recorded in the form shown in Fig. V, and those for mefloquine in a similar form (not shown here). The results can be entered in the graph sheet shown in Fig. VI.

(*m*) If the growth in the controls is adequate and the parasites are only *Plasmodium falciparum*, the following conclusions may be drawn in the case of blood samples containing less than 90 000 parasites per μl:

(1) Total inhibition of growth at 4.00 pmol chloroquine or mefloquine per well indicates susceptibility to standard chloroquine or mefloquine treatment.

(2) Growth at 5.7 pmol or more per well indicates resistance of *P. falciparum* to chloroquine or mefloquine.

(3) Growth at 4.00 pmol per well but inhibition at 5.7 pmol may still be compatible with a satisfactory response to the drug tested.

N.B.: The *in vitro* micro test is not meant to replace the *in vivo* assessment of the response to treatment with chloroquine. However, it provides a useful means of monitoring parasite sensitivity to drugs and is a convenient method of detecting the emergence of drug resistance.

Fig I PREPARATION OF THE BLOOD MEDIUM MIXTURE

(*a*) Draw 0 9 ml of growth medium into a 1 ml sterile disposable tuberculin syringe

(*b*) Inject growth medium into sterile holding vial

(*c*) Clean and firmly prick the finger of the patient selected

(*d*) Draw 100 μl of blood into a sterile anticoagulant-treated micropipette

(*e*) Expel the blood from the micropipette into the vial

(*f*) Close the vial with a sterile cap and swirl the vial to provide a homogenized suspension of blood

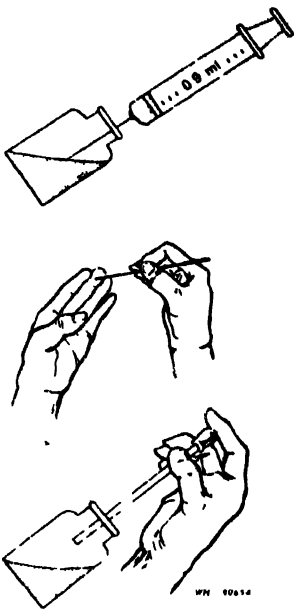


Fig II DOSING OF MICROPLATES

(*a*) Remove the predosed culture plate from the plastic bag using a surgical scalpel

(*b*) Remove the plastic seal from the columns according to the number of tests to be performed

(*c*) Remove the plate lid from the plastic bag and cover the plate

(*d*) Shake the blood/medium mixture gently to resuspend the blood cells in the vial

(*e*) Lift the lid and, with an Eppendorf pipette, add 50 μl of blood/medium suspension to each of the wells of the appropriate column, starting with well A and continuing to well H

(*f*) Replace the lid and shake the plate gently to reconstitute the drugs

(*g*) Write the patents' reference number on the lid over the appropriate columns

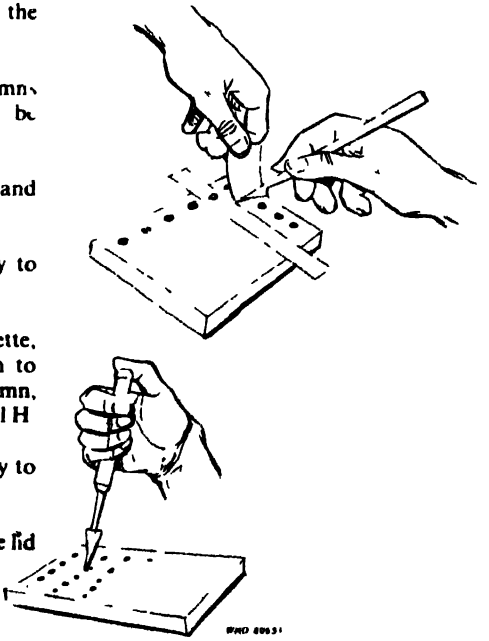
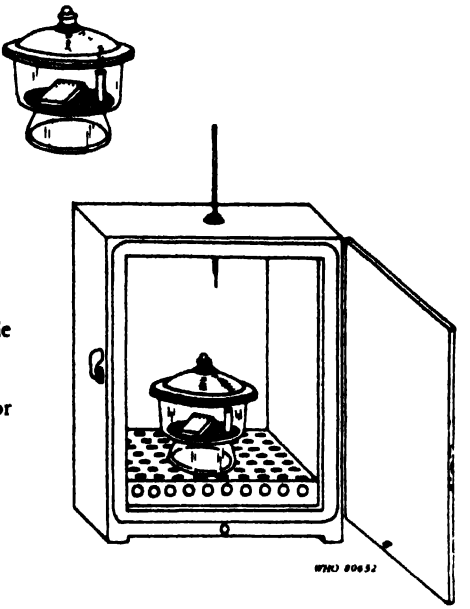


Fig III INCUBATION



(*a*) Place the plate in a candle jar

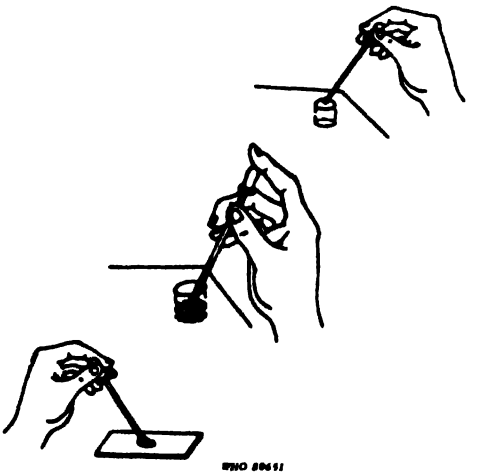
(*b*) Light the candle

(*c*) Replace the cover almost completely

(*d*) Replace the cover completely when the candle is almost extinguished

(*e*) Place the jar in an incubator at 37 38 C for 24 26 hours

Fig IV PREPARATION OF BLOOD FILMS



(*a*) Remove the plate from the incubator

(*b*) Take the plate out of the candle jar

(*c*) Using 50 μl (non-heparinized) micropipettes with a bulb, remove the growth medium

(*d*) Draw blood from the well into a micropipette

(*e*) Prepare a thick blood film

NOTE The micropipette tube must be changed for each well

Fig V FORM FOR REPORTING INDIVIDUAL RESULTS OF WHO STANDARD *IN VITRO* TESTS FOR THE RESPONSE OF *P FALCIPARUM* TO CHLOROQUINE AND MEFLOQUINE

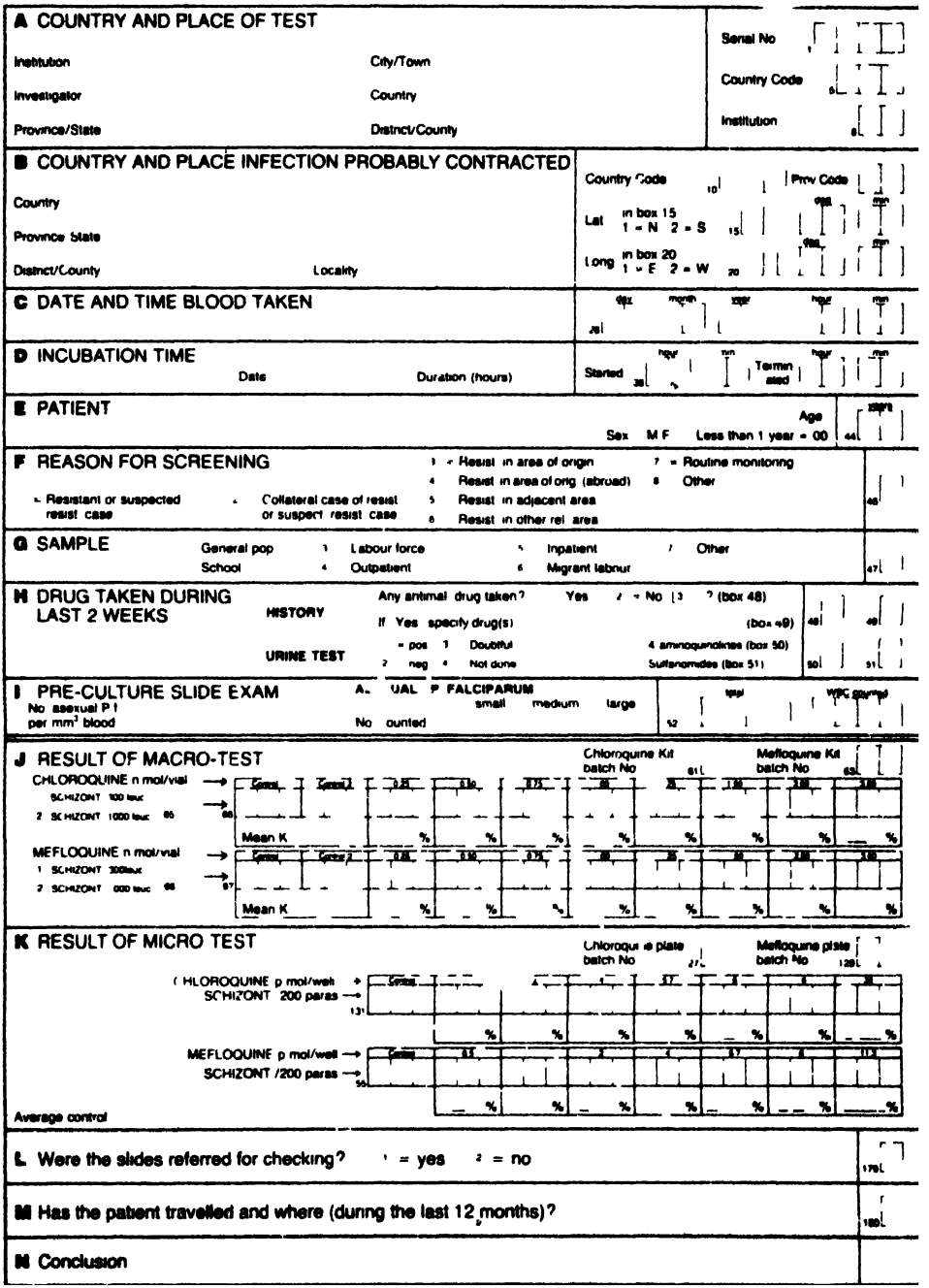
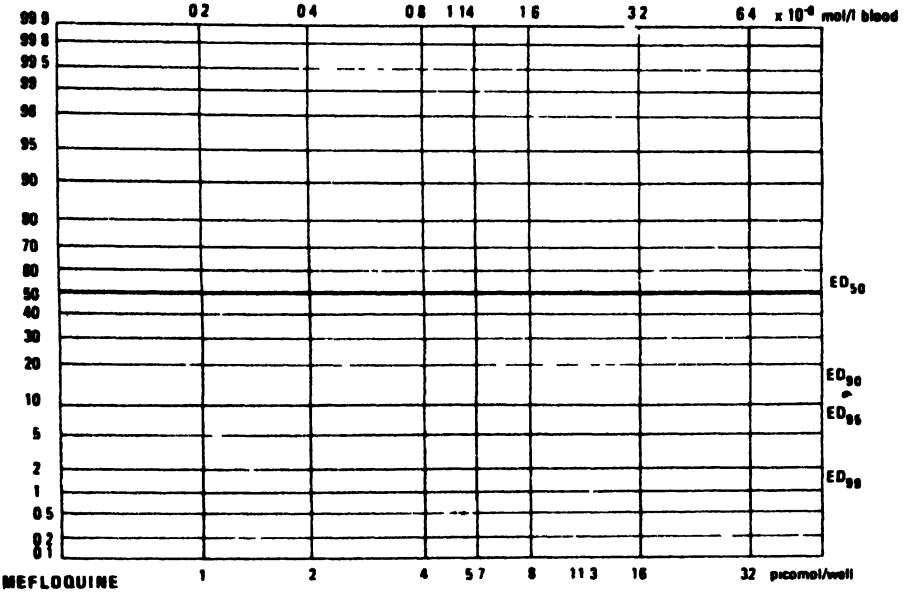


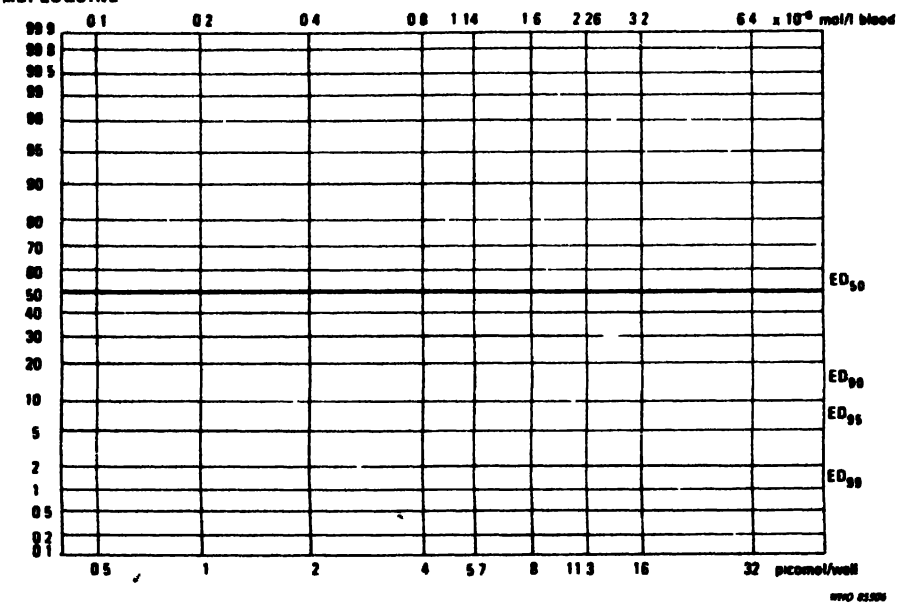
Fig. VI. GRAPH SHEET FOR *IN VITRO* MICROTEST FOR THE RESPONSE OF *P. FALCIPARUM* TO CHLOROQUINE AND MEFLOQUINE

PATIENT'S NAME:
DATE TEST STARTED
RECORD FORM NO..

CHLOROQUINE



MEFLOQUINE



Appendix to Annex 6

1 Relative molecular mass ("molecular weight") of compounds used in *in-vitro* sensitivity testing (related to base)

Quinine	324
Chloroquine	320
Amodiaquine	356
Proguanil	254
Pyrimethamine	249
Mefloquine	378

2 Conversion of substance concentration into μg l and vice versa, of selected compounds

Compound	To convert from mol/l into $\mu g/l$	To convert from $\mu g/l$ to mol/l
Quinine	324 × substance concentration × 10^6	$\frac{\mu g/l}{324} \times 10^{-6}$
Chloroquine	320 × substance concentration × 10^6	$\frac{\mu g\ l}{320} \times 10^{-6}$
Amodiaquine	356 × substance concentration × 10^6	$\frac{\mu g/l}{356} \times 10^{-6}$
Proguanil	254 × substance concentration × 10^6	$\frac{\mu g\ l}{254} \times 10^{-6}$
Pyrimethamine	249 × substance concentration × 10^6	$\frac{\mu g/l}{249} \times 10^{-6}$
Mefloquine	378 × substance concentration × 10^6	$\frac{\mu g\ l}{378} \times 10^{-6}$

ACKNOWLEDGEMENTS

The authors of this monograph acknowledge their debt of gratitude to all friends and colleagues who helped them in its completion, in particular to

Madame J Bernard-Kirukhine, Technical Officer, Parasitic Disease Programme, WHO, Geneva,

Dr J Haworth, formerly Chief, Office of Publications, WHO, Geneva, and previously Chief, Research and Technical Intelligence, Division of Malaria and Other Parasitic Diseases, WHO, Geneva

Dr T Lepeš, Director, Malaria Action Programme, WHO, Geneva and Mrs N Valabregue, Technical Officer, Malaria Action Programme, WHO, Geneva

Last, but not least, they thank the devoted secretaries and typists who prepared a series of drafts of the text

SELECTED BIBLIOGRAPHY

SELECTED BIBLIOGRAPHY

Preface

1 COVELL, G ET AL (1955) *Chemotherapy of malaria* Geneva, World Health Organization (Monograph Series, No 27)

2 WHO Technical Report Series, No 226, 1961 (*Chemotherapy of malaria*, report of a technical meeting), No 296, 1965 (*Resistance of malaria parasites to drugs*, report of a WHO Scientific Group), No 375, 1967 (*Chemotherapy of malaria*, report of a WHO Scientific Group), No 529, 1973 (*Chemotherapy of malaria and resistance to antimalarials*, report of a WHO Scientific Group)

Introduction and Historical Outline

3 BOYD M F, ed (1949) *Malariology* Philadelphia, Saunders, vol 1 2

4 COATNEY, G R (1963) Pitfalls in a discovery the chronicle of chloroquine *American Journal of Tropical Medicine and Hygiene* **12** 121 128

5 COATNEY, G R ET AL, (1971) *The primate malarias* Bethesda, United States Department of Health, Education, and Welfare, National Institutes of Health

6 DUNSCHEDE, H D (1971) *Tropenmedizinische Forschung bei Bayer* Dusseldorf, Michael Triltsch Verlag

7 GARNHAM, P C C (1966) *Malari parasites and other haemosporidia* Oxford, Blackwell

8 HARRISON, G (1978) *Mosquitoes malaria and man* London Murray

9 JANSSENS P G (1974) Le proces du paludisme *Journal of Tropical Medicine and Hygiene*, 77 (Supp), 47 53

10 JARAMILLO ARANGO, J (1950) *The conquest of malaria* London, Heinemann Medical Books

11 LEPEŠ, T (1972) Research related to malaria a review of achievements and further needs *American Journal of Tropical Medicine and Hygiene*, **21** 640 647

12 LEPEŠ, T (1974) Review of research on malaria *Bulletin of the World Health Organization*, **50** 151 157

13 MACDONALD, G (1973) In Bruce-Chwatt, L J & Granville, V J ed *Dynamics of tropical disease* London, Oxford University Press

14 PAMPANA, E J (1969) *A textbook of malaria eradication*, 2nd ed London, Oxford University Press

15 RUSSELL, P F (1955) *Man s mastery of malaria* London, Oxford University Press

16 SADUN, E H , ed (1969) Experimental malaria (a panel workshop) *Military Medicine*, **134** 729–1306 (No 10, special issue)

17 SADUN, E H , ed (1966) Research in malaria (An international panel workshop) *Military Medicine*, **131** (Supp) 847–1272

298. WERNSDORFER, W. H. (1980) Field evaluation of drug resistance in malaria; *in vitro* microtest. *Acta Tropica*, 37: 222–227.

Clinical and Preventive Use of Antimalarial Drugs

299. CHONGSUPHAJAISIDDHI, T. ET AL. (1979) Treatment of falciparum malaria in Thai children. *South East Asian Journal of Tropical Medicine and Public Health*, 10: 132–141.

300. DOBERSTYN, E. B. ET AL. (1979) Treatment of vivax malaria with sulfadoxine-pyrimethamine and with pyrimethamine alone. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 73: 15–17.

INDEXES

SUBJECT INDEX

A

D

E

F

G

H

I

N

O

Q

R

S

U

NAME INDEX

[redacted]

—The Lancet

[redacted] ISBN 81-7089-643-5 Code No. C 02566